Appropriate stimulant application to determine latex physiology character and production of clone RRIM 921 in Regency of Deli Serdang

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Abstract. Physiological characteristics of rubber trees are closely related to the ability to form latex. Sucrose, inorganic phosphate, and thiol levels in latex are physiological characters. The purpose of this study was to determine the correlation between physiological characters and latex production of clone RRIM 921 and proper stimulant application without disturbing the health of rubber trees. This research was conducted at PT. Perkebunan Nusantara III (Persero), Sei Putih Farm, and Physiology Laboratory of Sungei Putih Research Institute in 2018. The clones tested in this study were 17 years old of clone RRIM 921. This study uses correlation statistical analysis and regression test. Correlation analysis show that Pi level has negative significant correlation with Thiol latex, where high level of Pi will be followed by low level of thiol and vice versa. Latex production of clone RRIM 921 in May, June and July were not affected by latex physiology. The right stimulant application after leaf fall for RRIM 921 is in May.

1. Introduction
One of the rubber clones cultivated in Indonesia especially in North Sumatra is RRIM 921. The clones of RRIM 921 (Rubber Research Institute of Malaysia) are derived from cross breeding between Tjir 1 x PB 86. Such clone is low metabolism clone (LM), which has been cultivated since 1996, belong to superior clones because has high production potential namely 2,000 - 2,200 kg/ha and even reach 3,000 kg/ha when agroecological conditions support, wood producers and very sensitive to tapping panel dryness [1].

In North Sumatra, the area of rubber plantations (Hevea brasiliensis) in the period 2013-2016 has growth around 0.45 percent per year on average. In 2013, rubber planting area was 394,113.57 ha and increase to 394,519 ha in 2016. Regency of Mandailing Natal, Langkat, and Padang Lawas Utara were the centres of smallholder rubber plantations in North Sumatra [2].

Climate factors such as rainfall can affect the physiology of latex, growth and production of rubber trees [3]. Climate factors, especially rainfall, determine the physiological characteristics of latex and rubber production because increased rainfall means increase in sucrose and this is parallel with the development of leaves that are mature enough to photosynthesize [4-6]. Meanwhile, Dry Rubber Levels is determined by several factors such as clone type, rainfall, tapping time, and age of the tree.
High productivity can be determined by physiology character and latex production [7]. A number of researches have applied the concepts of latex physiological characteristics by looking at the initial conditions of metabolic activities of each clone to determine the proper exploitation system and predicting production potential according to clone ability [8,9], [10], [11] and [12].

2. Materials and methods

The experiment was conducted in two locations, namely at PT. Perkebunan Nusantara III Sungai Putih, and Physiology Laboratory of Sungei Putih Research Institute in 2018. First location was at Sungei Putih Research Centre, Indonesian Rubber Research Institute (3°25′33.8″N 98°52′04.8″E), and Sei Putih Estate, PT. Perkebunan Nusantara III (3°24′35.1″N 98°52′58.5″E). Both locations were located at Deli Serdang Regency, Province of North Sumatra, Indonesia. The soil type of the sites are ultisol and located around 25 m above sea level.

17 years old of clone RRIM 921 with planting space 2.5 m x 5 m was used as plant material in this study. Prior to the treatment, a randomized sampling was performed to select 75 trees with girth 65 cm - 70 cm.

Observation variables is physiological characteristics of latex, namely level of sucrose, inorganic phosphate and thiol which were measured using sample of TCA latex serum (Trichloroacetic acid). Latex serum is made by mixing 1ml of latex with 9 ml of TCA.

2.1. Yield and physiological parameters measurement

Fresh latex was collected in every tapping day. The yield (indicated dry rubber yield) was calculated by multiplying fresh yield with dry rubber content (DRC). Yield per tree per tapping (g t⁻¹ t⁻¹) was the result of total dry yield divided by number of tapped trees and number of tapping days.

Latex physiological parameters observation was performed at Sungei Putih Research Centre Laboratory. The measurement included sucrose, inorganic phosphate, and thiol contents. Trichloroacetic acid (TCA) serum was used for this analysis. Latex samples were collected 25 trees from 75 trees. The serum was made from 1 ml latex and 9 ml TCA prior to the analysis. All physiological parameters were measured using Beckman DU 650 Spectrophotometer (Beckman Instruments, Inc., USA).

2.2. Sucrose content

Latex samples were taken as much as 150 µL then added with 2.5% TCA so that the total volume is 500 µL. Then added with 3 ml of anthrone reagent and divortex, then heated by soaking in boiling water for 15 minutes then cooling. The next stage is absorbance at λ 627 nm (nanometer), then measured using the anthrone method. Sucrose dehydrated in concentrated sulfuric acid (70% H₂SO₄) and heating will give furfural derivatives which react with anthrone to produce blue colour. Absorbance measurements were carried out at λ 627 nm (nanometer) according to the anthrone [13].

2.3. Inorganic phosphate (FA) (mM)

Inorganic phosphate is measured based on binding principle by ammonium molybdate then reduced by FeSO₄ in an acid reaction to becomes blue in colour. Absorbance measurements were carried out at λ 750 nm (nanometer) using the [14].

2.4. Thiol (R-SH) (mM)

Latex samples were taken approximately 1.5 ml, and added with 2.5% TCA so that the volume was 1.5 ml, then added with a 10 mM DTNB 75 µL. Added with 1.5 ml of Tris 0.5 M buffer and divortex. Let it stand at room temperature for 30 minutes. The absorbance was read at λ 412 nm (nanometer) with a Beckman DU 650 spectrophotometer or measured from TCA serum based on the principle of reaction with dithiobis-nitrobenzoat acid (DTNB) to form yellow TNB which absorbed at λ 421 nm (nanometer) according to the [15].
2.5. Data analysis
This study uses primary data and secondary data. Primary data is field experiment. Correlation and regression test are used to determine the relationship between observation variables, while regression test to determine the shape of the relationship between physiological characters and latex production. Secondary data is rainfall data for 7 years (2012-2018).
Statistical analysis was conducted using the Minitab program ver. 18 to obtain the average value of each tree from each observation variable, and to get the middle value, minimum, maximum, standard deviation and coefficient of diversity from the components of latex yield.

3. Results and discussion

3.1. Agroclimatic condition
Rainfall conditions for 7 years, from 2012 – 2018, at the research site show monthly variations as presented in Figure 1. Oldeman’s climate classification for North Sumatra especially at Deli Serdang is Wet Month fell in May, dry months falls in January to March and other months fall into humid months [16].

Effect of latex yield is depending on the amount of rainfall, because climates such as rainfall, sunlight, air temperature, humidity, soil and wind greatly affect the physiology and latex yield. The highest rainfall is in May and the lowest is in March. Rainfall begins to decline from June to August, then rises again in September but not as high as May (Figure 1).

Number of Rainfall has crucial effect on physiology and latex yield. Increasing rainfall in May (Figure 01) will be followed by leaf development and latex yield will increases, so that the proper stimulants application time after leaf fall is starts in May.

![Figure 1. Distribution of rainfall from 2012 – 2018](image-url)
Climate factors such as rainfall, sunlight, air temperature, humidity, soil and wind greatly affect the physiology and latex yield. Groundwater as an important element in photosynthesis and nutrient solvent comes from rainfall because photosynthesis depends on ground water availability and heat energy for ambient temperature coming from sunlight. [16] stated that minimum rainfall required for rubber trees is 1,500 - 2,500 mm/year. [17] stated that rubber trees will grow optimally if cultivated in an environment that has the following climate: (1) rainfall around 2000 mm/year or more, evenly distributed without interspersed with dry months, with around 125 to 150 rainy days per year; (2) air temperature is 20°C to 34°C with a monthly average of 25°C to 28°C; (3) air humidity is around 80% with moderate wind speed; (4) high sunlight intensity for around 2000 hours per year with exposure time of 6 hours per day throughout the month.

**Figure 2.** The frequency curve for latex Yield (g/p/s) in clone RRIM 921

**Figure 3.** The frequency curve for latex Yield (g/p/s) in clone RRIM 921

**Figure 4.** The frequency curve for latex Yield (g/p/s) in clone RRIM 921
Latex yields were higher in May (Figure 2) and June (Figure 3) when compared with latex yield in July (Figure 4). This is because effect of rainfall on new trees was seen after 1 month later. Effect of low rainfall (in June) can be seen after one month later that is latex yield is low in July. [18] states that if ground water content is still available to plants and the environmental conditions support it, photosynthesis will run. In fact, in May, water reserves still available in the soil (higher rainfall in May compared to June) and high sunlight to supports photosynthesis process, in turn latex yield appear to decrease in July 1[19]. Long dry period causes water status in plant tissue cells decrease (low) because water evaporated through transpiration is greater than water absorption by plant roots [20]. [21] suggested that at low temperatures and precipitation, photosynthetic will be low and the growth rate was also slow.

3.2. Character of clones RRIM 921 without stimulants

Simple statistical analysis on physiology observations and latex production are summarized in Table 01. The Table shows that physiological characteristics of latex for three months (May, June and July) has coefficient variation (Coef.Var.) among the observed variables. The highest coefficient of variance was found at latex sucrose level, followed by thiol and Pi levels, namely 33.18%, 28.47% and 21.78%, respectively. This shows that sucrose levels are more influenced by environment (33.18%) than Pi levels (28.47%) and thiol (21.78%). Sucrose content is the main ingredient in latex formation where for three months observation (May, June and July), leaf observation is in full condition. According to [22], [4] and [23], sucrose levels is more influenced by seasonal variations compared to exploitation systems.

| Variable | Sucrose (mM) | Pi (µM) | Thiol | Rubber Yield (g/p/s) |
|----------|--------------|---------|-------|---------------------|
| **May**  |              |         |       |                     |
| Minimum  | 2.84         | 5.16    | 0.11  | 2.20                |
| Mean     | 8.77         | 9.69    | 0.36  | 4.48                |
| Maximum  | 5.23         | 6.64    | 0.27  | 2.93                |
| Median   | 0.60         | 0.51    | 0.03  | 0.25                |
| Coef. Var (%) | 33.18 | 21.78  | 28.47 | 24.80              |
| **June** |              |         |       |                     |
| Minimum  | 2.84         | 5.16    | 0.11  | 1.94                |
| Mean     | 8.77         | 9.69    | 0.36  | 4.77                |
| Maximum  | 5.23         | 6.64    | 0.27  | 2.89                |
| Median   | 0.60         | 0.50    | 0.03  | 0.29                |
| Coef. Var (%) | 33.18 | 21.78  | 28.47 | 28.02              |
| **July** |              |         |       |                     |
| Minimum  | 2.84         | 5.16    | 0.11  | 1.62                |
| Mean     | 8.77         | 9.69    | 0.36  | 4.36                |
| Maximum  | 5.23         | 6.64    | 0.27  | 2.88                |
| Median   | 0.60         | 0.51    | 0.03  | 0.28                |
| Coef. Var (%) | 33.18 | 21.78  | 28.47 | 30.60              |

Variation coefficients of Pi and thiol levels are low, namely 21.78% and 28.47%. This means that the conditions of Pi and thiol are slightly influenced by environment. Pi level indicates metabolic activity in latex vessels whereas thiol act as antioxidants to suppress oxidative stress as result of active
metabolism in cells [24]. Low levels of Pi and thiol indicate that exploitation not intensive yet [8]. Such condition indicated that plants are still not in active metabolism (sub-optimum) because they have not been treated (exploitation system). Low metabolic activity is indicated by low Pi content and only low sucrose converts into rubber. The medium sugar content in the latex shows slow conversion of sucrose to rubber [12].

Table 02 shows correlation test among each variable observed for three months. Results of correlation analysis showed that latex physiological variables show not significant correlation in May, June and July. But the correlation between Pi and thiol latex in May, June and July was significantly different and negatively correlated, meaning that if biosynthetic activity of rubber increased (Pi), thiol in latex as antioxidant will lower, thus rubber experiencing high stress level and vice versa. The function of thiol is as an antioxidant, so that oxidative stress as a result of active metabolism in cells can be suppressed [24].

| Variables | Yield (g/p/s) | Sucrose (mM) | Thiol (mM) |
|-----------|--------------|--------------|------------|
| May       |              |              |            |
| Sucrose   | 0.56         |              |            |
| Pi        | 0.05         | 0.08         | -0.67*     |
| Thiol     | 0.11         | 0.33         |            |
| June      |              |              |            |
| Sucrose   | 0.52         |              |            |
| Pi        | 0.06         | 0.08         | -0.67*     |
| Thiol     | 0.15         | 0.32         |            |
| July      |              |              |            |
| Sucrose   | 0.44         |              |            |
| Pi        | -0.17        | 0.08         | -0.67*     |
| Thiol     | 0.50         | 0.33         |            |

Note: *) Significant at 0.05

4. Conclusion
Correlation analysis show that Pi levels has negatively correlated and significant effect on Thiol latex, that is higher Pi levels means lower Thiol level and vice versa. Latex yield of clone RRIM 921 in May, June and July are not affected by latex physiology. The right timing of stimulant application after leaf fall for clone RRIM 921 is in May.

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