

PLANT PHYSIOLOGICAL ASSESSMENT OF HIGH METABOLIC RUBBER TREE CLONE IN NORTH SUMATRA, INDONESIA

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Abstract. Plant physiological assessment of rubber tree (*Hevea brasiliensis*) was aimed for yield optimization opportunity. In this present study, we observed the physiological status of PB 260 clone, a high metabolic, and the most popular clone in North Sumatra Province, Indonesia. The study covered three main rubber producer regions namely Asahan, Deli Serdang, and Labuhan Batu in 6 six different age of the plants. The physiological assessment was performed through latex diagnosis with four parameters i.e. total solid content (TSC), sucrose content, inorganic phosphorus (Pi) content, and thiols (RSH) content. The analysis results showed that there were no significant differences in TSC, Pi, and thiols contents in observed regions, while sucrose content in Deli Serdang was significantly lower than in Labuhan Batu, while in Asahan was not significantly different with other two other regions. The average sucrose value in Labuhan Batu, Asahan, and Deli Serdang were 5.82 mM, 5.74 mM, and 3.20 mM respectively. According to our analysis, PB 260 clone in Labuhan Batu had a good physiological condition, suggesting that the tapping intensity might be increased to optimize the yield. In Asahan, yield optimization should be performed prudently, whilst in the Deli Serdang region tapping intensity needed to be reduced due to poor physiological status. The harvesting system adjustment should be carried out carefully as we found a variety of physiological status among plant age.

Keywords: *Hevea brasiliensis*, latex diagnosis, harvesting, clone

INTRODUCTION

North Sumatra is one of the major natural rubber producer provinces in Indonesia. Rubber cultivation has been practiced in North Sumatra for more than a century and has become the main source of income for the community. In 2017, this province produced 460,901 tons of dry rubber (Directorate General of Estate Crops of Indonesia, 2018). A suitable agroecosystem is the main advantage of this province for rubber (*Hevea brasiliensis*) cultivation. This perennial species is harvested through tapping activity, incise the stem bark to allow the latex to flow out. Various products can be generated from natural rubber processing.

The latex harvesting system in *H. brasiliensis* is arranged to optimize the clonal potency. Latex diagnosis (LD) is a method to figure out plant physiological conditions through assessment of latex biochemical compounds including total solid content (TSC), sucrose, inorganic phosphorus (Pi), and thiols (RSH) contents. This method can be used for clonal character identification related to tapping system response (Jacob *et al.*, 1995; Gohet *et al.*, 2003) and plant physiological monitoring for tapping system adjustment (Adou *et al.*, 2017; 2018). By knowing the clonal typology and plant physiological status, planters can increase tapping intensity to get a high yield or

reduce it to avoid over-exploitation. The LD method has been widely used in scientific works, yet in a commercial rubber plantation, the adoption of this technology is still limited.

In this present study, we observed plant physiological status through latex diagnosis in commercial plantation extent of PB 260 clone, a high metabolic and the most popular clone in North Sumatra. The observation was aimed to determine the opportunity for yield optimization. This study might also be a consideration for LD application in other cultivated clones.

MATERIAL AND METHODS

The observation was carried out in North Sumatra Province, Indonesia, covering three main rubber producer regions in this province namely Asahan, Deli Serdang, and Labuhan Batu. The climatic characteristics of these regions were comparable with the annual rainfall of more than 2,000 mm which is favorable for rubber cultivation (Table 1). In each region, latex diagnosis was performed to PB 260 clone, the most popular clone cultivated in these regions, including 6 six different age of plant namely 7, 8, 9, 12, 13, and 14 years old. Three different blocks were used as replication.

Table 1
The rainfall and number of a rainy day in the observed region in 2020 (January – October)

Month	Rainfall (mm)			Rainy day (days)		
	Asahan	Deli Serdang	Labuhan Batu	Asahan	Deli Serdang	Labuhan Batu
January	153.33	101.75	115.75	10	6	5
February	112.67	69.75	57.25	9	6	4
March	46.67	53.00	33.75	5	5	4
April	169.33	251.00	140.25	10	11	10
May	277.33	342.00	243.50	14	13	13
June	358.67	223.50	337.75	13	10	13
July	411.33	374.50	240.00	18	14	16
August	288.67	186.00	195.00	13	7	12
September	154.67	81.50	256.00	13	7	14
October	321.33	315.25	322.50	23	15	16
Total	2,294.00	1,998.25	1,941.75	128	93	106

The physiological assessment was performed through latex diagnosis with four main parameters i.e. total solid content (TSC), sucrose content, inorganic phosphorus (Pi) content, and thiols (RSH) content. The TSC measurement followed the standard ASTM-D 1076-02 (ASTM International, 2002). For sucrose, Pi, and RSH quantification, sample serum was prepared from 1 ml latex and 9 ml of trichloroacetic acid (TCA) 2.5%. The sucrose content procedure was performed according to Dische (1962). Sample serum (150 µl) was added by TCA 2.5% up to 500 µl of total volume, then added by 3 ml of anthrone reagent. Following 15 minutes of soaking in boiling water and rapid cooling in freshwater, the absorbance was measured at λ 627 nm.

Inorganic phosphorus (Pi) content was assessed per Taussky and Shorr (1953). Samples serum (2.0 ml) was added with 2.0 ml ferrous-sulfate solution and incubated for 5 minutes at room temperature. The absorbance was measured at λ 750 nm. The RSH determination procedure was adopted from Mc Mullen (1960). The TCA serum (1.5 ml) was added by 75 μ l of 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB) 10 mM and 1.5 ml Tris buffer 0.5 M. After 30 minutes of incubation at room temperature, the absorbance was measured at λ 412 nm.

Data analysis was performed using XLSTAT statistical software (Microsoft Corp., Washington, USA). Analysis of variance (ANOVA) was performed for all physiological parameters. Least squares means were compared based on the Tukey comparison method at $\alpha = 0.05$.

RESULTS AND DISCUSSION

Total Solid Content

This parameter showed the proportion of solid compounds contained in latex, including rubber particles. Total solid content (TSC) indicated the level of biological activity and the balance between rubber biosynthesis and water importation (Jacob *et al.*, 1995; Gohet *et al.*, 2019). A low of TSC value reflects inadequate latex regeneration due to high tapping intensity (Jacob *et al.*, 1989). On daily basis, the TSC value can be used as the estimator for dry rubber content (DRC) where 90% of latex TSC referred to the DRC value.

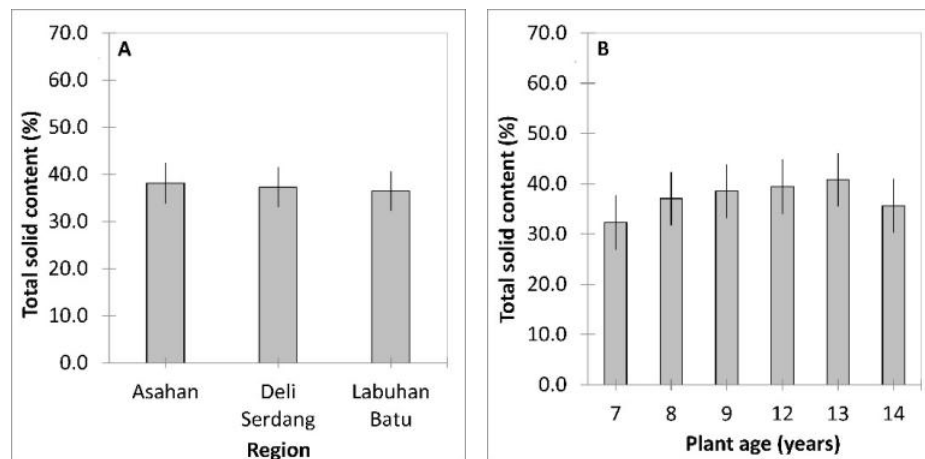


Fig. 1. Total solid content by region (A) and plant age (B). Error bar: confidence interval

Our analysis showed that the TSC value in three observed regions was comparable 38.14%, 37.26%, and 36.43% in Asahan, Deli Serdang, and Labuhan Batu region respectively (Fig. 1A). The TSC values indicated that the tapping intensity on the PB 260 clone in three observed regions was quite similar. In this present study, we detected an increase of the TSC, though not statistically significant, along with the age except in the 14 years of plant age (Fig. 1B). The TSC value in the young PB 260 rubber tree (7 years old) was 32.27%, while from 8 to 13, the TSC values gradually increased by 37.06% - 40.75%. The increase of leaves volume and root system by age

might give the advantages to intensify rubber biosynthesis in older trees. Our result was in line with Rachmawan and Sumarmadji (2007) that reported a linear correlation between DRC and age. Interestingly, the TCS decrease in the oldest observed age (14 years old), indicating that the plant might be received a higher tapping intensity.

High-quality products of rubber, such as ribbed smoked sheet (RSS) and centrifuged latex, require a certain minimum rubber content (Maspanger, 2007; Morshed *et al.*, 2018). As the high tapping and stimulation intensities lead to a TSC decline (Dian *et al.*, 2016), The harvesting regime in the field should take the TSC parameter into account. Nonetheless, to figure out the plant physiological status, the TSC value should be confirmed by other physiological parameters including sucrose, inorganic phosphorus, and thiols contents.

Sucrose Content

Rubber particles are biosynthesized through the mevalonate (MVA) and 2-C-methyl-D-erythritol 4-phosphate (MEP) pathways (Chow *et al.*, 2012). The sucrose content parameter exhibits the availability of rubber raw material as sucrose is the carbon source for rubber biosynthesis (Tupy, 1985). Together with inorganic phosphorus (Pi) content, sucrose is the main indicator for yield optimization through stimulation adjustment (Gohet *et al.*, 1996).

Statistical analysis revealed that the sucrose content in Deli Serdang was significantly lower than in Labuhan Batu, while in Asahan was not significantly different from the other two regions (Fig. 2A). The average sucrose value in Labuhan Batu, Asahan, and Deli Serdang were 5.82 mM, 5.74 mM, and 3.20 mM respectively. In this observation, we observed the PB 260 clone which naturally possesses low-medium sugar loading (Gohet *et al.*, 2019). According to the sucrose values, the yield optimization might be applied in Asahan and Labuhan Batu, while in Deli Serdang, a reduction of tapping intensity is required. In terms of the plant age, all sucrose content in six different ages showed a range of 4.30 mM – 5.32 mM without any significant differences among them. A mild increase of tapping intensity might be applied in mature plants especially 9 – 14 years old trees.

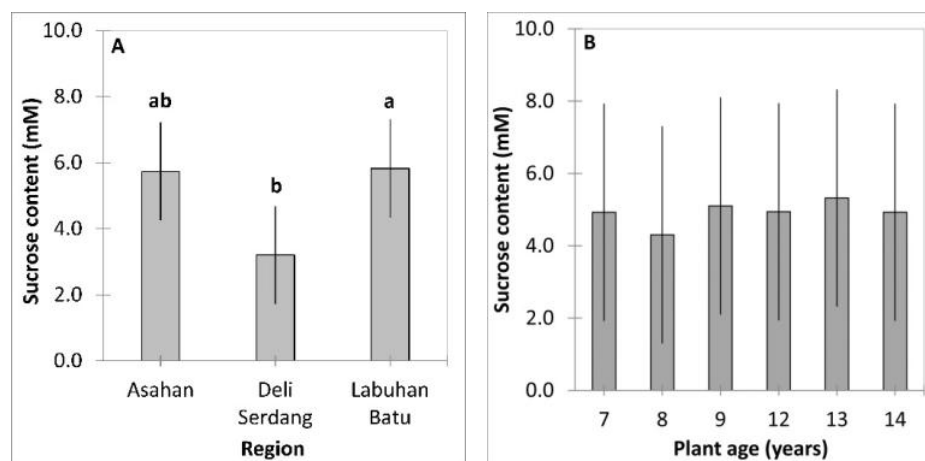


Fig. 2. Sucrose content by region (A) and plant age (B). Error bar: confidence interval

Sucrose content is a pivotal parameter in latex diagnosis as sucrose not only provides material for rubber biosynthesis but also the energy for other crucial metabolic pathways including antioxidant biosynthesis. A naturally low sugar loading leads to the high susceptibility of oxidative stress. Some studies reported that PB 260 is susceptible to tapping panel dryness (TPD), a physiological syndrome induced by over-accumulation of reactive oxygen species (ROS) (Okoma *et al.*, 2011; Putranto *et al.*, 2015).

Inorganic Phosphorus Content

Cellular metabolic activities including rubber biosynthesis require energy. Inorganic phosphorus (Pi) content shows the level of energy in the laticiferous system (Gohet *et al.*, 2019). High inorganic phosphorus content indicates that the plant spent a lot of energy on rubber particle regeneration, while a low value indicates that the trees have not been exploited optimally. Together with the sucrose content, these two parameters are required for stimulation adjustment.

Our observation showed that there was no significant difference in Pi content between region (Fig. 3A) and plant age (Fig. 3B). Inorganic phosphorus content in Asahan, Labuhan Batu, and Deli Serdang regions were 12.40 mM, 11.18 mM, and 10.47 respectively. A variation of inorganic phosphorus content was encountered among plant age, the value ranged from 8.54 mM to 13.42 mM, yet not statistically different. A low inorganic phosphorus content suggested that the metabolic activity in the laticifer was still low, thus, the yield can be optimized through stimulation adjustment. A study by Atsin *et al.* (2017) showed that the application of ethephon stimulation increased the inorganic phosphorus value.

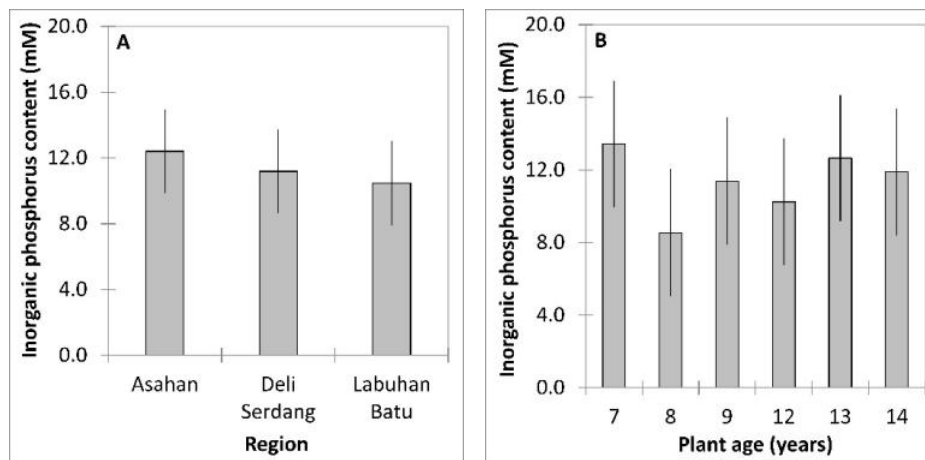


Fig. 3. Inorganic phosphorus content by region (A) and plant age (B). Error bar: confidence interval

The Pi value partially confirmed the sucrose content. Generally, a negative correlation occurs between inorganic phosphorus and sucrose content as low inorganic phosphorus indicating the limited metabolic level in the laticiferous system leads to the sucrose accumulation. Our observation found this relation in the Labuhan Batu region, while in the Deli Serdang region, low values were encountered in sucrose content and

inorganic phosphorus parameters, suggesting that other factors might inhibit the photosynthesis process.

Thiols Content

The organo-sulfur compounds that contain a carbon-bonded sulfhydryl group (R-SH), so-called thiols have important roles in the stress responses of plants due to their redox properties (Rouhier *et al.*, 2015). Thiols (RSH) content reflexes the physiological status of the plant. A low value of RSH content indicates that the plant is under stress.

The present study showed that RSH content was comparable in the three observed regions (Fig. 4A), namely 0.51 mM in Asahan, 0.54 mM in Deli Serdang, and 0.57 mM in Labuhan Batu. In different plant ages, we found a variation in RSH content, though statistically different. A high RSH value was found in young trees (7 years old) of the plant with the RSH value was 0.77 mM. The low RSH contents were encountered in 8, 9, 12, 13, and 14 years old of the plants, ranged from 0.43 mM to 0.58 mM. The low thiols content encountered in this study might be due to the physiological character of the PB 260 clone, which typically has low sugar loading. Sucrose is the energy source for various cellular metabolism including antioxidant regeneration.

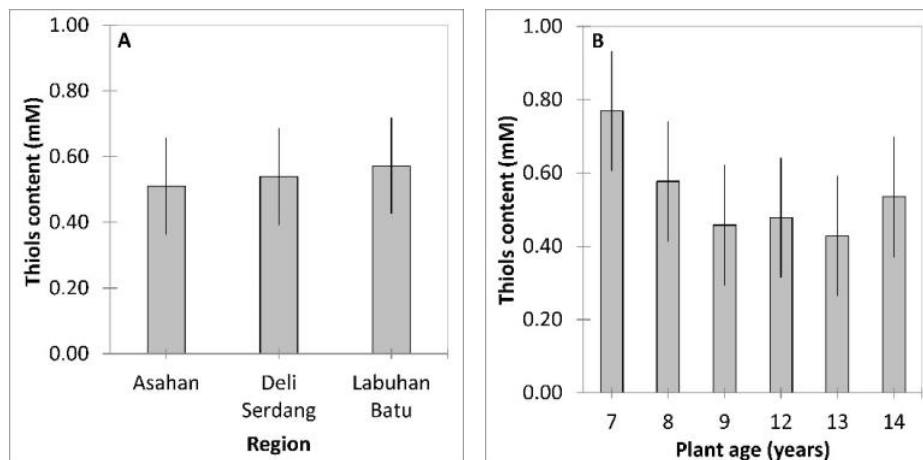


Fig. 4. Thiols content by region (A) and plant age (B). Error bar: confidence interval

H. brasiliensis harvesting practice, through bark incision and exogenous ethylene application, leads to reactive oxygen species. Antioxidants play important roles to maintain redox homeostasis in the laticiferous system. In this observation, we found that the plant of 9, 12, and 13 years possessed very low thiols contents. This condition might be due to the high metabolic activities, reflected by high inorganic phosphorus content.

Through a comprehensive deliberation of four latex diagnosis parameters, we can identify that PB 260 clone in Labuhan Batu had a good physiological condition, showed by high TSC, sucrose, and RSH contents, while the Pi value was low, suggesting that the tapping intensity might be increased to optimize the yield. In

Asahan, the TSC, sucrose, and Pi contents were high, yet the content of the thiol was low, suggesting that the yield optimization should be performed prudently. Latex diagnosis in the Deli Serdang region showed that the reduction of the tapping system is required due to low sucrose and thiols contents. The harvesting system adjustment should be carried out carefully as we found a variety of physiological status among plant age. In this observation, we could not detect the significant effect of climatic factors as the regions observed had comparable climatic characteristics (rainfall and rainy day).

CONCLUSION

Latex diagnosis allowed us to monitor plant physiological status. Based on our observation, tapping system intensification in PB 260 clone could be applied in Labuhan Batu and Asahan region, while in Deli Serdang region the tapping intensity needs to be reduced. The tapping intensity modification needs to be carried out wisely since plant physiological status might vary among plant age. This approach can be used as a consideration for harvesting system adjustment and can be expanded for other cultivated clones.

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