Sucrose analysis in leaf tissue of two contrasted rubber tree clones under abiotic and biotic stresses

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Abstract. The sucrose content in rubber leaf is assumed to differ between genotypes and environmental conditions. We studied the relation of sucrose-content and concentration between high- and low-latex metabolism clones, such as PB 260 and PR 300, respectively under biotic- and abiotic-stresses (white root disease and tapping panel dryness). The experimental design was a complete randomized design of two treatments (by genotypes and by stress conditions) with five replicates. Sucrose analysis was performed using the anthrone colourimetric modified method at 627 nm of spectrophotometry. Statistical analysis used the Student Newman-Keuls test of ANOVA at 5% significant level. The different types of latex metabolism clones had not affected the value of sucrose-content and concentration in leaf tissue. By contrast, the results showed that WRD-affected trees of PB 260 clone (24.45 mg g\textsuperscript{-1}) had significantly the highest sucrose content compared with healthy- (15.53 mg g\textsuperscript{-1}) and TPD-affected trees (13.42 mg g\textsuperscript{-1}). A similar trend was equally observed in TPD- (16.33 mg g\textsuperscript{-1}), healthy- (14.51 mg g\textsuperscript{-1}) and WRD-affected trees (14.52 mg g\textsuperscript{-1}) of PR 300 clone. The sucrose-content and concentration in PB 260 clone at WRD-affected trees were the highest than the others.

1. Introduction

Natural rubber (\textit{cis}-1,4-polyisoprene) is commercially used as a raw material of various industrial products. It is synthesized in the cytoplasm of highly differentiated cells of the cambium namely laticifers [1, 2]. Latex containing natural rubber particle is harvested by tapping the outer layer of bark truncating the laticifers [2, 3]. The latex flows out directly after the first tapping until the beginning of coagulation processes by lutoid activity inside the laticifer cells [3, 4]. In periodical tapping, latex regeneration and duration of latex flow are critical factors reflecting latex production in rubber plantation [5]. Latex regeneration takes at least 48 hours to convert sucrose into \textit{cis}-1,4-polyisoprene in natural rubber biosynthesis [6, 7]. Latex regeneration between the two intercepts of tapping period is regulated by primary mechanisms associated with the sucrose availability, biochemical reaction providing energy, enzyme activity, water supply, nitrogen availability, and anti-senescence activity [6, 8-14]. A carbon and energy supply for latex regeneration is provided by sucrose derived from rubber tree leaves [15].

Sucrose is an early precursor for the biosynthesis of rubber particles [16]. The activity of latex vessel depends on the availability of sucrose inside laticifers [17-19]. The synthesis of isoprene
molecule requires the sucrose to be broken down into glucose and fructose by the invertase enzyme [20]. These processes generate the biochemical energy to be used for producing rubber particle [18, 21, 22]. The synthesis process begins with the breakdown of sucrose into other molecules through more than twenty enzymatic reactions to generate the first isoprene molecule of isopentenyl diphosphate (IPP) [23, 24]. IPP is a direct intermediate precursor for latex biosynthesis. It is a major derivative of the MVA (mevalonate) pathway or MEP (2-C-methyl-D-erythritol-4-phosphate) as an alternative biosynthetic pathway [23]. Both of these latex biosynthetic pathways occur in laticifers and require sucrose as the precursors [24]. The entry of sucrose into laticifers and enzyme activity are two important steps in latex synthesis [25]. In addition, sucrose and potassium play a role in regulating the turgor pressure (0.9-1.5 MPa) in the laticifers and surrounding cells in the bark to maintain the latex flow [26, 27]. Meanwhile, sucrose in the cytoplasm is used for metabolite activities evaluation equally present in the laticifers [11, 22].

Laticifers is an active sink to accumulate sucrose from photosynthesis organ. The sucrose transport into the laticifers is actively carried out throughout the plasma membrane [11, 17, 22, 27, 28]. The physiological and molecular regulation of sucrose metabolism has been widely studied in latex association to natural rubber production in laticifers [6, 18-20, 29]. Sucrose loading into laticifer is one of the limiting factors of latex production in Hevea brasiliensis [9, 30]. High sucrose-content inside the laticifers indicates that this precursor is not utilized for latex biosynthesis causing later reduced latex production [6, 7]. The sucrose availability supposes to depend on the photosynthesis capability in leaves. However, studies of the sucrose metabolism in rubber tree leaves are limited. Logically, to regenerate latex, a high level of sucrose-content in photosynthesis organ must be maintained. The sucrose in rubber leaf is assumed to differ between genotypes and environmental conditions. Photosynthesis process is inhibited when rubber trees undergo stress conditions disturbing the translocation of sucrose into laticifers. This research aimed to study the relation of sucrose-content between high- and low-latex metabolism of rubber tree clones under abiotic and biotic stresses (tapping panel dryness-TPD and white root disease-WRD).

2. Materials and Methods

2.1. Plant material
Leaf samples were obtained from rubber tree clones PB 260 and PR 300 (Figure 1) grown at the field trial of Cimons, Indonesian Research Institute for Biotechnology and Bioindustry, Bogor, Indonesia (Latitude: S 6° 36' 22.98''; Longitude: E 106° 45' 42.47''). PB 260 is a quick starter rubber tree clone as opposed to a slow starter PR 300. The samples were collected in three conditions such as healthy trees, tapping panel dryness (TPD)-affected trees at 33% of latex halt [25], white root disease (WRD)-affected trees carrying early visual attack symptoms. TPD as a physiological disease represents abiotic stress while WRD caused by Rigidoporus lignosus represents biotic stress. The collected samples were carried into the laboratory on dry ice and directly used for sucrose analysis.

2.2. Sucrose analysis
2.2.1. Anthrone reagent
The sucrose-concentration was measured using the modified anthrone colourimetric method [31]. This reagent was prepared by dissolving 0.1 g of anthrone (Merck, USA) in 100 mL of H₂SO₄. The acid was previously made by adding 100 mL of acid to 30 mL of water. The reagent was allowed to stand for 30-40 min with occasional shaking until complete clearance of the mixture. The reagent was freshly prepared each day and used within 12 hr.
Figure 1. Leaf samples of two contrasted rubber tree clones. (Left) A quick starter clone (PB 260). (Right) A slow starter clone (PR 300).

2.2.2. Sucrose standard
Sucrose stock solutions (1 mM) was prepared by dissolving 1.73 mg of sucrose powder (Caisson Labs, USA) in 125 μL trichloroacetic acid (TCA) 2.5%.

| Solution                      | Series of sucrose-concentration (mM) |
|-------------------------------|--------------------------------------|
| Sucrose stock solution (1 mM) | 0 0.01 0.025 0.05 0.1 0.2 0.5       |
| TCA 2.5% (μL)                 | 500 495 487.5 475 450 400 250       |

2.2.3. Method
Briefly, 0.5 g leaf tissue samples were extracted in a 50 mL centrifuge tube, and 10 mL of 80% ethanol was added. The samples were heated in a water bath at 85 °C for 30 minutes followed by a centrifugation at 10,000 rpm for 15 minutes. The supernatant was collected. The extraction was repeated twice (10,000 rpm for 15 min each) and heated in the boiling water until it has condensed to 5 mL. Distilled water was added to a final volume of 50 mL. Fifteen μL of the solution was taken followed by a sequential addition of 135 μL of distilled water and trichloro-acetic acid 2.5% (TCA) to a final volume of 500 μL. Finally, 3 mL anthrone reagent was added into the solution followed by incubation in boiling water for 15 min. The absorbance value at 627 nm was measured using spectrophotometer (Multiskan Go, ThermoScientific).

2.3. Data analysis and statistical
The sucrose-concentration (mM) for each of samples determined by the sucrose standard equation formula: 

\[ y = 2.3852x + 0.0235 \]

with ‘x’ is the absorbance data values. The sucrose-content value was calculated by the equation:

\[
\text{Sucrose-content (mg g}^{-1}\text{)} = \left( \frac{C \times V \times M_w}{W} \times F_d \right)
\]

where:
- C is sucrose-concentration obtained by referring to the standard curve (mM), V is the volume of solvent (L), Mw is sucrose molecular weight (342.3 g mol-1), W is the weight of the sample (g), Fd is the dilution factor (10x).

The mean values of sucrose-concentration obtained from five replicates of each treatment. Statistical analysis was performed using the Analysis of Variance (ANOVA) at 5% level followed by Newman-Keuls Student test (XLStat, Addinsoft, USA).
3. Results and Discussion

3.1. Sucrose-concentration in leaf and latex of two rubber clones

The results of sucrose analysis on rubber tree leaves were measured in mM for the sucrose-concentration and in mg g$^{-1}$ for sucrose-content values. The results showed that the sucrose-concentration in the rubber leaves was not influenced by genotype differences in tested clones used in this study. The sucrose-concentrations were measured at 0.052 mM and 0.044 mM in PB 260 and PR 300, respectively (Figure 2a). In the rubber tree, the sucrose parameter determined in latex is known to be associated with the biosynthesis of rubber particles. The sucrose-concentration in latex is the difference between sucrose-derived sucrose and the amount of sucrose used for latex metabolism [6, 18, 28]. The value of sucrose-concentration reflects the presence sucrose influx for usage in latex regeneration and/or latex metabolism inactivity where the process of reforming sucrose to rubber particle has not been intensive in laticifers [6, 8]. The study of Lizawati showed that the interaction of the sucrose-concentration in latex, especially at the interaction of the scion and rootstock of PR 300 clone was lower (1.060 mM) than the PB 260 clone (1.820 mM) [32, 33] (Figure 2b). Both studies used the anthrone colourimetric method of Dische [31]. This method is commonly used to measure the concentration of sucrose in the latex of rubber trees.

The genotype differences in latex metabolism between PB 260 and the PR 300 clone were first believed to be due to the difference of latex sucrose-concentration. The PB 260 clone is a quick starter clone having a high metabolism of latex production while the PR 300 clone is a slow starter clone with low latex metabolism [34, 35]. Herlinawati and Kuswanhadi [36] also reported that the PB 260 clone has a quick starter clone having high latex production in the first tapping. At the beginning of tapping, this PB 260 clone directly converted the sucrose with or without chemical stimulant for latex biosynthesis [6, 36]. The stimulant treatment tends to reduce the availability of sucrose in the leaves or latex. Logically, it leads to an understanding that the sucrose has been converted into rubber particles [6, 10]. The sucrose-concentration in latex PB 260 clone has decreased by increasing the frequency of tapping [37-39]. In PR 300 clone, both in the leaves and latex, the sucrose-concentration was lower than in PB 260. One hypothesis mentioned that in the slow starter clones, the sucrose is probably used for the formation of bark and stem tissue whose bark is thicker than quick starter ones [12, 35, 40, 41].

![Figure 2](image-url)  
*Figure 2. The sucrose-concentration in PB 260 and PR 300 clones (a) leaf (b) latex* (*data from Lizawati [32] and Woelan et al [33]).

According to Sumarmadji [6], the high value of sucrose-concentration in rubber tree has not significantly described latex production. It is due to the sucrose-concentration not having a direct correlation from its potential latex productivity. Nevertheless, there is a critical limit on the availability of sucrose-concentrations in laticifers during latex synthesis. Sumarmadji and Tistama [42] stated that the threshold value of sucrose-concentration was about 4 mM. Increasing tapping intensity can reduce the sucrose-concentration value under the threshold so that it will induce the void of latex compiler precursor. Other studies showed similar results for the value of the critical limit of sucrose-
concentrations in latex [37]. The sucrose-concentration below 5 mM is a crucial point in latex biosynthesis for determining the pattern of rubber tree planting. The dynamics of sucrose-concentration values in latex were strongly influenced by the metabolic rate inside the whole plant tissues [9]. The range of sucrose-concentration values in mature trees at the beginning of tapping was 3.75-6.91 mM [37]. The low sucrose-concentration indicates the asynchronous metabolism of photosynthesis results in the rubber trees development stage [18, 43]. By contrast, the high sucrose-concentration means less active metabolism. The high concentrations of sucrose can also indicate that the laticifer cells are no longer functioning or degenerating [35, 38, 44]. In this paper, both in leaf and latex, the sucrose-concentration cannot directly describe the actual high production in two contrasted rubber tree clones.

3.2. Sucrose-concentration of two contrasted rubber tree clones under biotic and abiotic stresses

The results of this study showed that the value of sucrose-concentration in the leaf samples varied toward interactions of rubber clone and plant conditions. The value of sucrose-concentrations in WRD-affected trees of PB 260 clones was significantly higher (0.072 mM) compared with healthy (0.046 mM) and TPD-affected trees (0.043 mM) (Figure 3). This could be explained by the hypothesis that plant defence system induces a stress signals so that the leaves are forced to perform photosynthesis faster before senescing. WRD causes early yellowing of leaf colour in rubber tree. This mechanism is due to the extraction of the chlorophyll before leaf fall [45]. The low value of sucrose-concentration in TPD-affected trees showed the correlation between sucrose influx and sucrose content in laticifers. Moreover, sucrose is supposed to be used for actively repairing bark tissue previously degraded by TPD. Jacob and Krishnakumar [46] stated that the incidence of TPD occurs due to physiology disturbances in latex biosynthesis and the process after the formation of rubber particles. These mechanisms lead to the anatomy damage of latex vessel tissues.

![Figure 3](image-url)

Figure 3. The sucrose-concentration in leaf samples of two contrasted rubber tree clone (PR 260 and PR 300) on healthy-, WRD- and TPD-affected trees condition. WRD: white root disease; TPD: tapping panel dryness. The bars with different letter are significantly different according to the Newman-Keuls test of XL-STAT with the p-value of 0.05.
TPD-affected trees have contained lower macro and micronutrients both in latex and bark compared to healthy trees [47]. In PR 300, the sucrose-concentration values were not significantly different in healthy trees (0.044 mM), WRD-affected trees (0.043 mM) and TPD-affected trees (0.048 mM) (Figure 3).

PR 300 having a low latex metabolism in laticifers is more tolerant to TPD. The sucrose is actively used in the recovery process of bark tissue damage. However, the sucrose is not a dominant factor in determining TPD occurrence, since sucrose may not be converted into latex during TPD [6]. According to Gohet et al. [48], latex production and developmental aspects such as tree growth actively were actively in competition in the use of sucrose as a precursor of metabolic processes. The allocation of sucrose to laticifer is based on the balance between produced and appropriated sucrose [17, 22].

3.3. Sucrose-content in the leaves of PB 260 and PR 300 clone under WRD and TPD

The content of sucrose in leaves is directly supposed to be equal to the sucrose-concentration value. In PB 260, the sucrose-content in WRD-affected trees was significantly higher (24.45 mg g⁻¹) than healthy (15.53 mg g⁻¹) and TPD-affected trees (13.42 mg g⁻¹) (Table 2). These results suggest that the sucrose which has been produced in photosynthesis process is suspected to be in-transmitted into laticifers for latex biosynthesis in WRD-affected trees. In addition, the early infection of WRD could be identified in the leaves damaged by the senescence. Consequently, the leaf-vessel cells do not optimally work. These conditions commonly show changes in the foliage colour, flowering, and prematurely pollination [45, 49, 50]. Tree branches affected by WRD infections will degrade until the entire canopy is destroyed and eventually the rubber tree dies [49, 50]. On the contrary, it can be observed that the sucrose-content in the leaf was relatively low in TPD-affected trees because this physiological disease occurs uniquely in the rubber tree soft bark. The sucrose can still be transported from leaves into laticifers. The PB 260 clone is a high metabolic clone with low sucrose-content in laticifers due to the continuous use of sucrose as a precursor of latex biosynthesis [51, 52]. Thus, PB 260 clone is well known to be susceptible to TPD [47, 53]. In TPD-affected trees, the sucrose is suspected to accumulate higher in the laticifers because it cannot be used as a raw material for latex biosynthesis as the result of latex vessel damage caused by TPD [41, 46, 54, 55].

The high intensity of TPD occurrence is frequently found in clones with high metabolic of latex production such as PB 260 as opposed to low metabolic clones such as PB 217 and PR 300 [41, 53]. The sucrose-content values in PR 300 clone were not significantly different in healthy (14.51 mg g⁻¹), TPD- (16.33 mg g⁻¹), and WRD-affected trees (14.52 mg g⁻¹) (Table 2). In a low metabolic clone such as PR 300, the flow of sucrose translocation from the leaf to laticifers is supposedly slow.

| Table 2. The sucrose-content value obtained from the calculation of sucrose-concentration standardized by the sucrose standard curve. The value is compared with 0.5 g leaf samples in each treatment. |
|----------------|-----------------|------------------|
| Rubber tree clones | Rubber tree condition | Sucrose-content (mg g⁻¹) |
|----------------|-----------------|------------------|
| PB 260 | Healthy trees | 15.53± 1.33 |
| | WRD-affected trees | 24.45± 9.90 |
| | TPD-affected trees | 13.42± 6.83 |
| PR 300 | Healthy trees | 14.51± 1.45 |
| | WRD-affected trees | 14.52± 1.98 |
| | TPD-affected trees | 16.33± 1.39 |

*The values with different letter are significantly different according to the Newman-Keuls test of XL-STAT with the p-value of 0.05
* WRD: white root disease; TPD: tapping panel dryness
It also suspected that the sucrose inside laticifers is saturated due to the relatively slow conversion of sucrose to rubber particle in latex biosynthesis [20, 28]. PR 300 clones are moderately tolerant to TPD. Thus, the photosynthesis process to produce sucrose in leaves normally works [38, 39, 56]. The high sucrose-content in the latex indicates good influx in laticifers. But, it can also be the consequence of low metabolism of sucrose conversion into rubber particle [6].

3.4. The clone PB 260 and PR 300 latex yield production

Based on the latex metabolic type, the rubber clones divided into low, medium, medium, high-and medium-high metabolism classes [52]. Commonly, high metabolic clones have higher velocities of latex biosynthesis compared to medium or low metabolic clones. The clonal metabolism type determines the pattern of latex production during tapping exploitation cycles [9, 11, 36, 38]. Physiologically, latex production is influenced by several important factors such as the latex recovery between two tappings and the duration of latex flow [5]. The PB 260 clone has a higher latex production than the PR 300 clone (Figure 4). It is caused by the difference in metabolic rate in both of clones to convert sucrose into rubber particles through several enzymatic stages in laticifers. PB 260 is categorized as high metabolic, whereas PR 300 is low metabolic clone to produce latex [35, 39, 57, 58]. The high metabolic rubber tree clones have a high initial production which decreases in mid-age of latex production. In contrast, the low metabolic clone is relatively stable during the whole period of tapping for more than 10 years [35, 39]. The latex productivity of rubber trees depends on several major factors such as the number of laticifers and metabolic activity. The physiological development of laticifers is influenced by the rubber tree clonal genotype and its hormonal interactions [9, 44].

![Figure 4](image)

**Figure 4.** The average of dry rubber yield of quick starter- (PB 260) and slow starter rubber tree clone (PR 300 and PR 300) during 9 years of tapping production [59, 60].

4. Conclusions

The sucrose-content is equivalent to the concentration value of sucrose in the leaves of the rubber tree. The genotype differences between two contrasted clones related to the latex metabolism did not affect the content and concentration value of sucrose in leaves. The interaction between the two rubber clones (PB 260 and PR 300) and the stress conditions (WRD- and TPD-affected trees) showed that only WRD-affected trees influenced the sucrose-content in the leaves of PB 260. This condition was not met in PR 300.
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