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Physiological status of high and low metabolism *Hevea* clones in the difference stage of tapping panel dryness

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Abstract. *Tristama R, Mawaddah PAS, Ade-Fipriani L, Junaidi. 2019. Physiological status of high and low metabolism* Hevea *clones in the difference stage of tapping panel dryness. Biodiversitas 20: 367-373.* Tapping panel dryness (TPD) caused productivity of rubber trees drop sharply. However, the increase of TPD stage has not been completely elucidated, especially in physiological aspects. TPD incident was higher occurred in high metabolism than low metabolism clones. The incident has been classified based on visual observation. This research aimed to explore the physiological characters to identifying the physiological changes of each TPD stage in the two metabolism types of rubber clones, IRR 42 (low metabolism) and IRR 118 (high metabolism). The physiological parameters such as thiol, Pi content and peroxidase activity were specifically in each clone and the tissue types (bark or laticifer). The physiological pattern of IRR 42 was a difference with that of IRR 118 for the increase of the TPD stages. Phosphate inorganic content in the latex and bark were a decline in the TPD affected trees, whereas the sucrose content was relatively constant. The pattern of changes of thiol content in IRR 42 was reverse with IRR 118, both in the latex or bark tissue. Peroxidases activity in the latex and bark negatively correlated with the TPD stage. The decline of Pi and peroxidase activity can be used to identify the TPD incident in rubber trees.

Keyword: *Hevea brasiliensis*, latex metabolism, physiological parameters, tapping panel dryness

INTRODUCTION

Tapping panel dryness is a physiological disorder in laticifer tissue of *Hevea brasiliensis* bark caused the bark does not produce latex partially even totally. This condition was induced by reactive oxygen species (ROS) accumulation as a negative effect of latex harvesting stresses called over-exploitation (Sumarmadji 2000). As known, ethylene application and wounding as consequence of latex harvesting enhance ROS either in the latex or the bark. Over-accumulation of ROS disturbs laticifer functions and in long-term leads tapping panel dryness (Zhang et al. 2017). For increase rubber yield by activation of a low latex metabolism, application of an ethylene releaser (ethephon) to the bark elongates duration of latex flow and enhances latex regeneration between two tappings (d'Auzac 1989). The production of rubber has led to many studies on redox reactions and ROS-scavenging systems in laticifers, and on the supply of antioxidants to protect the rubber polymer (Chrestin 1989).

TPD-susceptible *H. brasiliensis* clone was found to be related to some biochemical parameters, such as low sucrose and inorganic phosphorus contents (Putranto et al. 2015). Ethepone application and wounding as consequence of latex harvesting enhance ROS. TPD-affected trees had smaller number of laticifer vessels compared to healthy trees, suggesting a modification of cambial activity. The differential transcript abundance was observed for twenty-seven candidate genes related to TPD occurrence in latex and phloem tissues for ROS-scavenging, ethylene biosynthesis and signaling genes (Zhang et al. 2017). Some Ethylene Response Factor (ERF) genes functions suggested that these candidate genes should play an important role in regulating susceptibility to TPD (Putranto et al. 2015).

Physiological characters related to TPD tolerance were still limited. Every rubber clone has difference response to stimulation and tapping frequency, or both combination (Okama et al. 2016). Generally, a high tapping frequency and ethephon stimulation induced early TPD incident in a high latex metabolism clone and late one in a low latex metabolism clone (Andrianto and Tistama, 2014). Tistama et al. (2017a) had reported that sucrose content in the latex of high metabolism clone was lower than low metabolism clone, but the reverse for inorganic phosphate content.

Li et al. (2010) reported that the genes play important roles in related to TPD included that the production and scavenging of ROS, ubiquitin-proteasome pathway, programmed cell death, and rubber biosynthesis. In previously, several enzymes in the latex close related to TPD had been reported. High activity of NADPH oxidase and peroxidase were TPD symptom (Chestin 1989). Das et al. (1998) were reported that SOD activity in the bark which stimulated with high concentration of etephon exhibited lower than an untreated tree. The expression of SOD gen in the latex affected by ethylene treatment in a healthy tree but TPD did not affect the gene (Kongsawadworakul et al. 1997). In contrast, TPD affects the expression of glutathione reductase both in latex and bark (Deng et al. 2015), and also reported that relative

transcript abundance of small RNA *Hbpre-MIR159b* increased in the latex TPD affected trees (Gebelin et al. 2012).

Latex has several antioxidants which are involved in membrane protection from ROS, such as thiols, ascorbate, and γ-tocotrienol (Zang et al. 2017). Thiol is one of a physiological parameter in latex diagnostic as stress indicator. Low concentration of latex thiol indicates the rubber tree under exploitation, and the concentration increase followed to the exploitation intensity (Jacob 1989). When the rubber tree was in over-exploitation condition, thiol and Pi content in the latex was very high (Tistama et al. 2017b)

Tapping panel dryness in rubber tree can be minimizing at field stage with frequency and intensity tapping system (Chrestin et al. 1989; Senevirathna et al. 2007; Vijayakumar 2013). Kumari and Nugawela (2013) reported that rubber tree affected with TPD should be exploited lower tapping system. Some progress has been made in biochemical and physiological aspects, however the physiological process from the healthy trees to be TPD affected ones was poorly understood. The physiological status was usefully for classification of TPD stage that until now the TPD stage was classified using visually with measurement of the length of dryness line panel. The mostly, the study about TPD was the focus in the latex analysis. This study aimed to an observation of physiological status related to TPD stage in two metabolism types of rubber tree in its latex and bark.

MATERIALS AND METHODS

Plant materials

The descriptive research used two rubber tree clones were IRR 118 a high metabolism and IRR 42 a low metabolism. The two clones were planted closely in the same field that assumed had similar agroclimate. The plant was grown up in 2007 and tapped on bottom bark-two panels (B0-2). Each clone was classified into four criteria based on a percentage of the dried bark length; were 0% (healthy tree), 0-25%, 25-50%, and more than 50%. The mean of dried bark was the tapping line that did not exudate latex. The percentage of TPD has measured the length of dried bark divide total length of tapping strip times one hundred percent. Each class was replicated three times and each replication consisted of three plants. Latex samples from the three plants were collected into one tube. Bark samples were collected from the three plants using cork borer (diameter 0.5 cm) at five cm under the middle of tapping line. All the samples were put in the container box containing gel ice and carried out to the laboratory.

Physiological analysis

The physiological parameters such as sucrose, inorganic phosphorus and thiol contents were evaluated the content of sucrose, inorganic phosphorus (Pi) and thiol, on the clear serum called TCA-serum (trichloroacetic acid) that obtained after latex acid coagulation, respectively, by the Ashwell anthrone method (1957), the Taussky and Shorr molybdate ammonium method (1953) and the Boyne and Ellman acid dinitro-dithio-dibenzoic method (1972). The results are stated in millimole per liter of latex ($mmol.L^{-1}$). Bark samples were ground in the mortar with nitrogen liquid. One gram of bark powder was extracted in 9 ml TCA. Sucrose, Pi and thiol contents were measured on the clear serum called TCA-serum used the same methods with latex. Sucrose reflected the balance between sucrose consumption by the laticifer (for energy production and rubber biosynthesis) and sucrose loading from the apoplast into the laticifer. Pi indicated the level of available energy in the metabolic activity of the laticifer. Thiol indicated the level of lutoids protection and the stability of latex. Sucrose, Pi and thiol contents were expressed in millimoles per liter of latex ($mmol.g^{-1}$).

Protein extraction and peroxidase activity

Protein extraction was performed following of procedure Packeer-Mohamad et al. (2012). The soft tissue of bark (3-4 mm from cambium) was ground with liquid nitrogen and extracted in 0.2 M phosphate buffer, pH 6.5, containing 0.25% (v/v) Triton X-100 and 3% (w/v) polyvinylpyrrolidone (PVPP). The homogenate was centrifugated at 12.000 rpm, 4°C for 15 min. The supernatant was separated for protein quantification and analysis of peroxidase activity. Protein content was measured by Bradford methods using BSA as standard (Bradford 1976), and peroxidase activity was assayed according to Shannon et al. (1996).

Data analyze

R statistic software version 3.4.2. (R Development Core Team) in studio version 1.1.383 environment. The effect of TPD stage in each clone was tested using ANOVA and Tukey Multiple Comparison at alpha = 5%. Correlation between parameters was tested Pearson Correlation analysis.

RESULTS AND DISCUSSION

This observation found several interesting physiological changes related with the two clones types, which have a different physiological in each TPD stage and both in the latex or bark tissue (Table 1). The process of TPD incidents was observed on the low metabolism clone (IRR 42) and high metabolism clone (IRR 118). Phosphate inorganic content in the IRR 42 latex has higher than IRR 118, while thiol, sucrose and peroxidase content of both clones were not differenced. On the contrary, the content of thiol and Pi in the bark tissue was lower in the IRR 42 than IRR 118. IRR 42 peroxidase activity in the bark tissue was higher than IRR 118. Inorganic phosphate in the latex of IRR 112 was categorized normal, while in IRR 118 was categorized very low. The facts show that metabolism in the latex of the TPD-affected IIR 118 was lower than IRR 42. The latex Pi content can be used as an indicator for TPD stage. If the Pi content was under the range (15-18 mM) that indicated the tree had affected TPD.

Table 1. The average content of physiological parameters in the bark tissues and latex of TPD-affected rubber tree clones.

Clone	Samples	Thiol (uM)	P inorganic (mM)	Sucrose (mM)	Peroxidase (unit/minute)
IRR 42	Latex	0.351	18.69	4.77	0.306
IRR 118		0.295	6.95	4.63	0.298
IRR 42	Bark	2,773	0,917	25,64	10.5
IRR 118		4.842	1.564	27,06	8.08

There were variations of physiological parameters both in the bark or latex. The content of thiol and sucrose, and peroxidase activity in the bark tissue were higher than in latex, 7.9-16.4 times, 5.3-5.8 times, and 27.1-34.3 times respectively. On the contrary, the inorganic phosphate content in the bark was lower 4.4-20.4 times than in the latex. High Pi in the latex demonstrated that activity metabolism in the laticifer was very high, especially for latex metabolism. Phloem in the bark supported sucrose into laticifer tissue as raw material for rubber synthesis and energy (Jacob et al. 1989). Sucrose was also distributed to the all plant tissues using length transport systems (Taiz and Zeiger 2002), like other raw material of structural tissue or energy. It may be reason why the sucrose content in the bark tissue higher than in latex. The peroxidase in the bark tissue may correlate with tolerance TPD, whereas thiol was not directly correlated with TPD tolerance.

Das et al. (2002) reported that over-exploitation triggered free radicals and its scavengers in rubber tree. Increasing of tapping system induced system defense such as thiol, superoxide dismutase (SOD) and peroxidase. Thiol and peroxidase were included in the plant defense system as an antioxidant. Our research shows that the thiol content in IRR 42 latex declined in the higher of TPD stage, whereas in the IRR 118 latex, the thiol content rise in the higher of TPD stage. Peroxidase activity of IIR 42 latex was relatively flat in the first to third of TPD stage and the activity increase in the fourth stage. Peroxidase activity leaped three times in the third TPD stage and declined sharply in IRR 118 latex. The thiol content in the latex of low metabolism was substituted by peroxidase activity in the higher stage, but in high metabolism both antioxidant were complementary (Figure 1). In the previously reported, the thiol contains in PB 260 (high metabolism clone) were significantly lower in the TPD-affected trees compared to the healthy trees (Putranto et al. 2015).

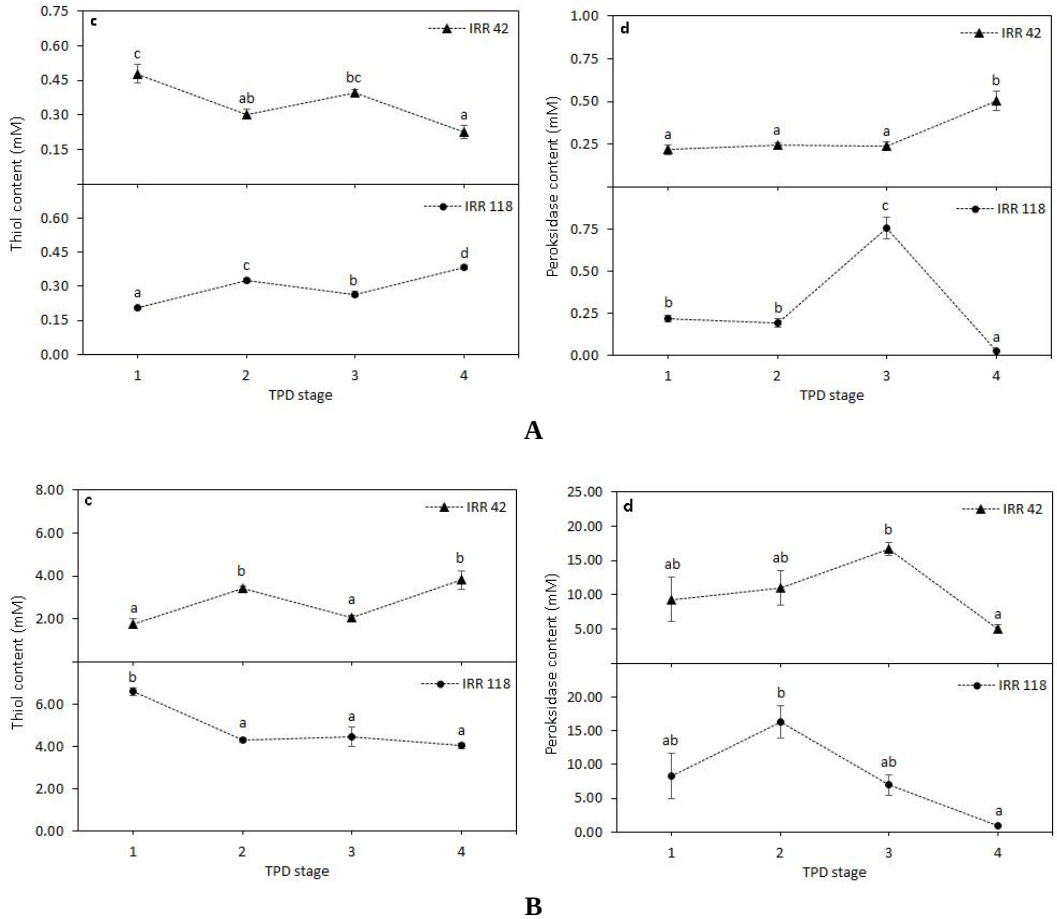

Figure 1. Physiological dynamic of thiol content and peroxidase activity in the different stage of TPD: A. Latex, B. Bark

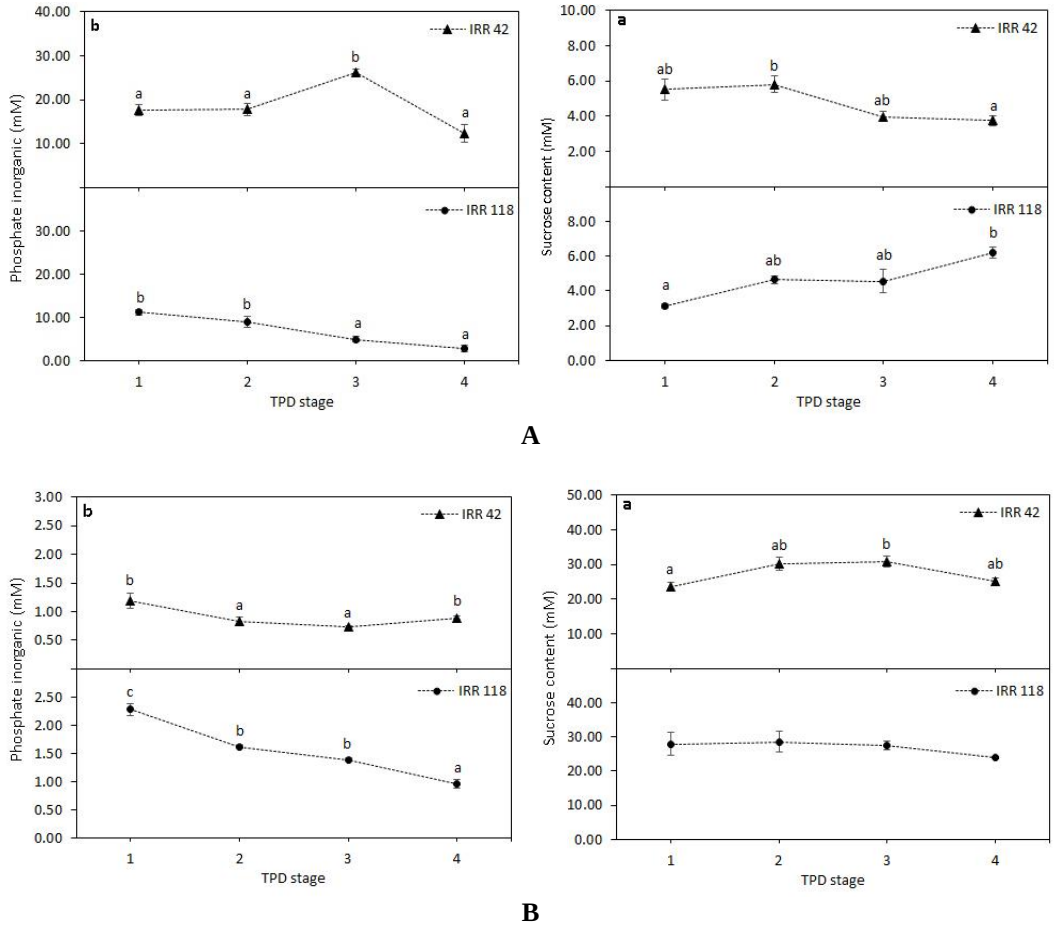

Figure 2. Physiological dynamic of Pi and sucrose contain in the different stage of TPD: A. Latex, B. Bark

The substitution pattern between thiol content with peroxidase activity also occurred in the bark tissue of IRR 42. In the IRR 118 bark tissue, both of antioxidant only rise in the lower TPD stage and then declined in the higher one. Refer to this phenomenon, the high thiols content and peroxidase activity in the bark and latex may be related to TPD tolerance in low metabolism clone. Xi and Xiao (1988) reported that the activity of peroxidase and superoxide dismutase decreased in a TPD-affected tree, however ascorbic peroxidase activity precisely increased in the partial TPD tree (Bapoetra et al. 2018).

Phosphate inorganic in the latex of IRR 42 was categorized as normal and flat in all of TPD stage. Sucrose contains as a raw material in low metabolism was also flat in all of TPD stage. Pi latex in IRR 118 dropped to below 5 mM after the third stage of TPD, while sucrose contains a decrease in higher stage of TPD (Figure 1). The raising of sucrose correlated with lowering of Pi in TPD incident. TPD incident decreased latex metabolism activity, especially rubber synthesis. In other words there was not any energy to converted sucrose to rubber particles, so sucrose was accumulated in the latex. The low Pi in the TPD-affected bark caused the increase of respiration for ATP supply. The other consequence of the low Pi was reducing of rubber particle size in the advanced stages of TPD (Krishnakumar et al 2001).

In the bark tissue, metabolism activity was lower than in the laticifer. The stage of TPD affected negative to metabolic activity in the bark IIR 118 but did not in IRR 42. Sucrose dynamic occurred in the bark of IIR 42, and they contain increase following the intensity of TPD. The was no significant change in sucrose contain in the bark of IIR 118 (Figure 2). Soluble sugars behaved like an intermediate, ready-to-use compartment in both wood and bark. In all tapped trees, the increase in storage occurred together with a reduction in trunk radial growth. This was interpreted as a shift in carbon allocation toward the creation of reserves, at the expense of growth, to cover the increased risk induced by tapping (Chantuma et al. 2009).

According to correlation analysis, the TPD stage has a negative correlation to inorganic phosphate content and peroxidase activity in the bark, 0.59 and 0.37 respectively. Pi in the bark supported the metabolism in the tissue, and metabolic activity in the bark has correlation 0.73 with thiol content in the bark. The thiol role was activator for the antioxidant enzymes which have a role in the several metabolism or transport system in the bark from free radical substances. The thiol contents in the bark and latex did not directly correlate with TPD stage. Interestingly, thiol content in the latex has a high negative correlation (-0.77) with thiol content in the bark. It was suggested, the thiol was transported from the bark to the latex or the reverse side for the changes of TPD stages.

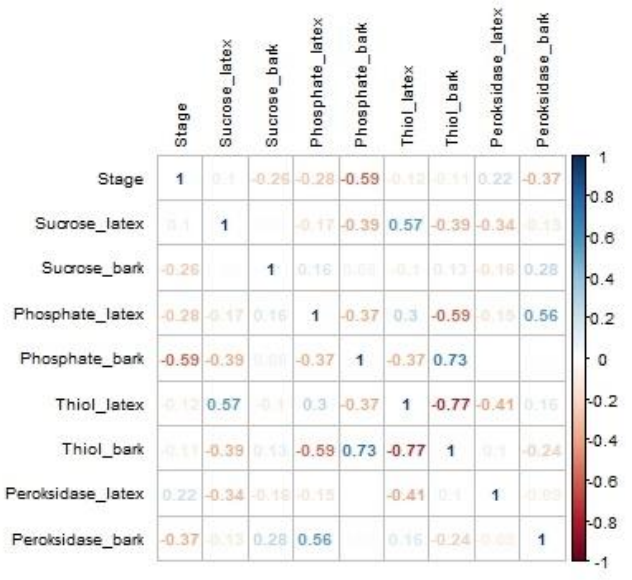

Table 2. The correlation analysis of the physiology parameters with the TPD stage

Figure 3. Performance of healthy tree, which is the wholely of line cutting exudate latex (*left*), and partial TPD affected tree, which is partially lined cutting exudate latex (*right*)

Bark Peroxidase has high correlation with Pi latex (0.56). The peroxidase scavenged the H_2O_2 or organic hydroperoxide between plasmalemma and the cell wall, or outside of cell wall laticifer. This activity may reduce ROS both in the bark and latex. Thiols have importance for enzymes activation in earlier TPD (second stage), and the next TPD stage, the antioxidant function was change by peroxidase. Peroxidase protected the plasmalemma, whereas thiol protected the enzyme associated with latex metabolism. Genes related to TPD were suggested producing stress/defense response and metabolism (Li et al. 2010). Compared with healthy tree latex, the genes associated with defense response were largely upregulated in the TPD-affected tree. Montoro et al. (2018) reported that analysis of differentially expressed genes were lower total of genes in TPD-affected tree than under exploitation tree. Jasmonic acid synthesis was importance pathway related TPD tolerance.

In other hands, the gene associated with metabolism and energy were largely downregulated (Li et al. 2010). Inorganic phosphate contents in the latex and bark have negative correlation to TPD stage. The decline of Pi content in the latex was sharper than in the bark, especially in the IRR 118. The low Pi caused the metabolisms diminished in both tissue, it was included rubber biosynthesis. This reason answered why sucrose contents were relatively stable in all of TPD stage. Alkaline/neutral invertase (A/N-Invs) is key enzyme that hydrolysis sucrose in an initial step of rubber synthesis in the laticifer (Liu et al. 2015). The A/N-Invs activity was one of enzymes in the rubber synthesis pathway that may be interfered by ROS.

Overexpression of SOD gene increase SOD activity in rubber tree leaves, and activated all ROS scavenging enzymes (Leclercq et al. 2012). Plant transgenic-POD was significantly higher tolerance to H_2O_2 than wild-type plant (Teng et al. 2016). The high concentration and activity of antioxidant enzymes in the plant tissue were proved enhancing tolerance to stress such as over-exploitation. These characters were potential as maker for TPD incident stage and TPD tolerance in rubber tree.

The previous research, accumulation of polypeptide was occurred in the latex of trees-affected TPD. Accumulation was caused the release rubber elonging factor (REF) and small rubber particle protein (SRPP) from rubber particle into cytosol (Sookmark et al. 2002), and can be used for TPD marker. The TPD-sensitive clone had higher cyanogenic potential and showed the quicker and stronger responses to the cyanogenic compounds (de Fay et al. 2010). The cyanogenic compounds also induced hislogical abnormality such as tannin cell, in situ coagulated latex and tylosoid (de Fay et al. 2010). Venkatachalam et al. (2009) identified gen which correlated with TPD in the bark tissue. The *HbTOM*20 gene (*H. brasiliensis* translocase of the outer mitochondrial membrane) expression was significantly down-regulated in the bark of TPD-affected trees compared to a healthy one. The other gene, The *HbMyb*1 is likely associated with TPD and that the function of *HbMyb*1 is associated with the integrity of bark tissue of rubber trees (Chen et al. 2002).

In this research, each rubber clones has specifically pattern of physiological parameter changes. *H. brasiliensis* clones have not the same level sensitivity to tapping panel dryness and have specifically defense mechanisms. (Okama et al. 2011) classified rubber clones based on sensitivity to TPD into three groups, the sensitive clones, intermediate clones, and tolerance clones. All recommended rubber clones should be investigated advance its change patterns, that use to develop the understanding of TPD tolerance mechanism. This study is important for rubber tree breeder in creating of the new rubber clones that tolerance for TPD.

In conclusion, physiological status in the rubber clones was specific depending on the type of metabolism and the TPD stage. Inorganic phosphate content dropped in both metabolism types and tissue, and caused the low of metabolism in the TPD-affected trees. The decrease of metabolic activity in IRR 118 affected TPD was higher than IRR 42. Thiol content closely correlated with metabolism activities, especially in the bark. Peroxidase activity has a negative correlation with TPD stage. This enzyme was interesting to deeply investigate its correlation with TPD tolerance clones.

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