Physiological Respon of Tpd-affected Rubber Plant to Growth Regulator and Antioxidant Treatments

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Abstract: The research aimed to determine bark and latex the effect of applied NAA on thiol content, total protein and peroxidase activity in the bark and latex of TPD –affected rubber tree (*Hevea brasiliensis* muell Arg.). The research was conducted at the Sungei Putih Estate with 54 meters altitude, in leaf fall session from January 2018 to July 2018. The experiment was design using Split Plot Design, with three factors i.e: clones (RRIM 921 and PB 260), condition of plants (Healthy and TPD) and the formula (control, 2.5% and 5% the stimulants). Parameter observed were thiol and protein content and *peroxidase activity*. The results showed that the range of values bark and latex are Thiol 0.1-1.7 µM, Protein 31-124 ppm and Peroxidase 0,01- 0,9 unit/ppm protein, the clone of rubber plants was significantly affected the thiol's. The NAA application could improve the physiological characters of the rubber plant and the activities from all parameters significantly affected by environmental conditions.

1 INTRODUCTION

Production of rubber plants with potential production decreased reaching 114.74 kg / ha / t and 183.05 kg / ha / year caused of Tapping Panel Dryness (TPD) (Mochlisin & Tistama, 2014). In Indonesia, TPD incident on productive rubber estate reach 20-50%, which is characterized by the cessation of latex flow and dryness for of the line of tapping. The economical lossing caused by TPD incident are estimated to be more than 1.7 billion/ year. Not balancing in latex metabolism caused exploitation and the less maintenance of plants are as mean factors inducing TPD incident (Andryanto & Tistama 2014). Efforts to limited incident are manuring, and restoration of tapping fields for 6 months, with hormon application and triadimephone 250 g/l (Sirait Syahnen, 2013). However, TPD incident is still to be major problem in Indonesian rubber plantations.

Physiological stress caused tapping in rubber tree induces free radical compounds such as 0_2-, H_20_2, OH*, QO* (Jacob, 1989). These reactive system oxygen (ROS) enter into latex vessel cells (laticifer) and interfere laticifer cell organels that are causing cells disrupstion (Tistama, 2013). In TPD affected plant, many genes related with a role in triggering programmed cell death (PCD) were up regulated during the early of TPD syndrome (Venkatachalam et al. 2007).

Therefore, the earliest symptoms of histology appearing in laticifer cells. So, it's very likely to be said that the trigger for a oxidative stress was known in initially laticifer plants (Jacob and Krishnakumar, 2006). Joseph (2006) reported that the nutrient content of P, Mg, and Mn in the latex is higher, while Fe and Zn are lower in TPD-affected plant compared to normal plants. Gebelin *et al*., (2013) said detoxification of ROS by SOD, peroxidase and catalase activity or through other non-enzymatic mechanisms capable to detoxifying ROS from the tissue without causing disrups. Thus, peroxidase and catalase activities are key role in the process of removing H_2O_2 molecules in plant tissues.

Thiol in the plant tissue actives enzymes that have control in respons to environmental stress conditions as major antioxidace in the Hevea latex Zhang et al. 2017). Thiol levels are importance as indicatitor that is related to the physiological susceptibility of latex, especially in the incidence of TPD, and thiol status shows of plants to the pressure of exploitation (Zhang

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et al. 2017) The higher the intensity of exploitation the lower the thiol level (Sumarmadji *et al*, 2004). Sumarmadji and Tistama (2004) found thiol has the ability to protect sub-cellular organelles and to cacth the toxic oxygen molecules. This toxic oxygen molecule causes exhaustion of latex vessel cells which triggers TPD. The optimal content of thiol ranges from 0.4 to 0.9 mM and the critical condition is below 0.2 mm.

Peroxidase enzyme is one of the plant enzymes that has a relationship with the process of resistance (Zhang et al, 2017). According to Andrianto & Tistama (2014) the difference in tolerance between high and low metabolism of rubber clone is thought to be due to the content of antioxidant compounds in bark tissue and latex. To find out the sensitivity and resistance of plants to disease attacks an approach is used regarding the influence of environmental stress on plant physiology processes. such as ethylene treatment (Putranto et al. 2015).

Based on the early description, TPD is one of the important problems that immediately need to be resolved. The existence of leaf fall session can also reduce the value of rubber production. Research on rubber bark is needed cause the bark is where latex is produced. Analysis of several physiological parameters, is expected to be an early indicator of healing process. These study aimed to increase the cure rate of TPD by giving NAA formula and Ascorbate acid and in the future it can reduce production losses due to TPD and leaf fall session and to examine changes in antioxidant compounds in healthy and affected TPD plants with NAA and ascorbic acid nutrition treatments.

2 MANUSCRIPT PREPARATION

2.1 Page Setup

Plant materials used for this study were PB 260 clones (High metabolism) and RRIM 921 (Medium metabolism) planted in 2006 with 3 x 6 m spacing. The experiment was arranged with a Split-Split Plot Design with three replications, each replication consisting of 1 plant. The selection of the TPD affected plants was carried out by cutting the tapping panel with a tapping knife. Rubber plants are tapped using a system tapping 1/2S d/3 (tapped every 3 days with a length of 1/2 spiral of stem).

Formula for treatment in the form of minerals follows the composition of Murashige & Skoog (MS) with the addition of NAA (100 ppm, and ascorbic acid (150 ppm). The solution was spryed on tapping panel every 15 days for 3 month with a dose of 5 ml / tree. Latex samples were collected into steril tube, while bark samples were taken by cork borer (diameter 1 cm) at 5 cm under tapping line, then carrying in the box ice to laboratory.

2.2 Protein Extraction and Peroxidase Activity

According to Bradford (1976), protein extraction for lateks was perfomed by ten ml of each latex samples were centrifuged at 15000 rpm for 20 min at temperatur 4^{O}C. Clear phase was transfered into the new tube and used to protein and enzyme analysis. Protein extraction for bark 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). Bark samples were frozen in liquid nitrogen and grinded in the mortar. One gram of bark powders were extracted with phosphate bufer pH 7,2 using vortex for five minutes. The extracts were centrifuged at 12000 rpm in 4^{O}C for 15 minute. Supernatan was tranfered to new tube and ready for enzymatic analysis. peroxidase activity uses phenol-aminoantipirine A solution (phenol 810 mg solution and amino antiphirine 25 mg in 50 ml water) and solution B by mixing 30% H2O2 added with MES Buffer solution pH 6 measured at 510 nm absorbance (Shannon et al., 1996).

2.3 Physiological Analysis

For bark physiological analysis, one gram powder was extracted in TCA 5% solution, then suspension was centrifugated 10000 rpm for 15 minute. Analysis of thiol content was based on the principle of its reaction with dithiobis-nitrobenzoate acid (DTNB) to form a yellow TNB absorbed at λ 421 nm (nanometer) with a Beckman spectrophotometer DU 650 (Bobbiliof, 1923). Thiol indicated the level of lutoids protection and the stability of latex. Thiol contents were expressed in millimoles per liter of latex ($mmol.g^{-1}$).

2.4 Statistic Test

The results were tested by ANOVA and for the treatment effect will be continued with Tukey Multiple Comparison at alpha = 5%. Correlation

between parameters was tested Pearson Correlation analysis using Minitab version 16 sofware.

3 RESULT AND DISCUSSION

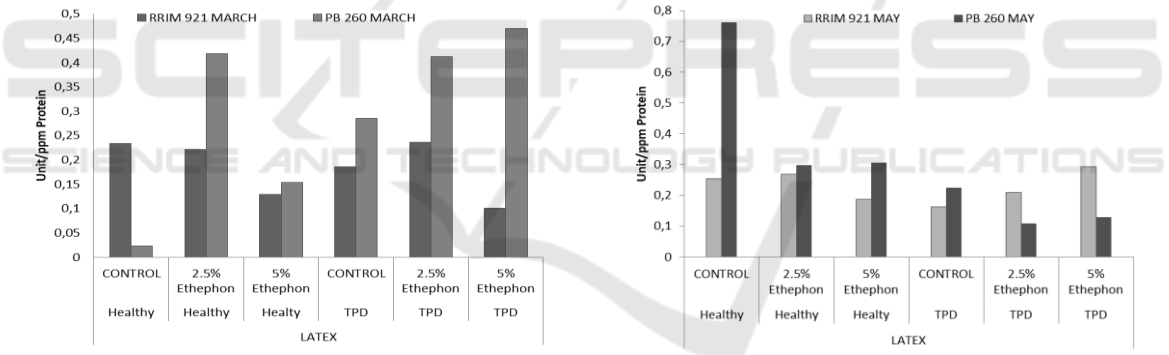
3.1 Result

The latex thiol content in the both of rubber clone (Figure 3) were lower in TPD affected plant than health plants. PB 260 was higher thiol content both in health or TPD partial than that of RRIM 921. In leaf fall condition, latex thiol contents were 2-5 times higher than full leaf condition. The thiol content was decrease in health clones after the trees were treated with ethepon. In TPD affected plants of PB 260, the thiol content was categorized high and its was not affected by ethepon treatments.In full leaf condition, the thiol contents was normally in all of plant healthy condition even in the plants treating with ethepon.

Generally, thiol content in bark of TPD affected plant the RRIM 912 was higher than that of healthy plants in March. The thiol content decrease in bark of this clone after treating with ethepon 2,5% and then increase with ethepon 5% treatment. In leaf fall condition PB 260 has diference pattern in its response to ethepon. Thiol bark content in May was relatively flate by ethepon treatment.

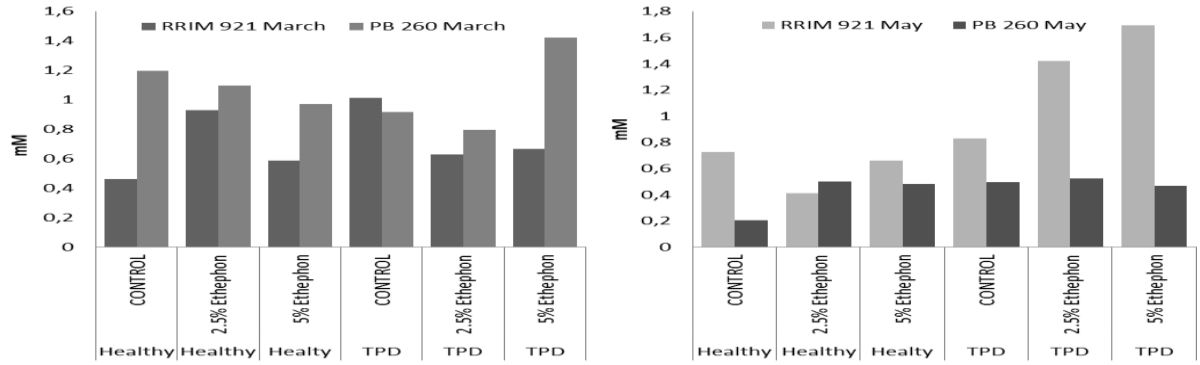
3.2 Discussion

Latex POD activity was affected significantly by leaf condition, ethepon treatment and clone. POD activity in the bark PB 260 in full leaf periode was very high in health plant, and the activity sharly reduced by ethepone treatments. The bark high peroxidase activity was negatively correlation with TPD level (Tistama et al. 2019). The level of POD activity was decrease by The health rubber tree could be indicated by high POD activity in the latex and bark both in low or high metabolism clone, however the level of POD activity should be classified based on level of physiology healthy. Opponent with POD activity, the thiol content in March or in fall leaf condition. In May when the leaf is full the thiol content decreased both in the latex and bark. The thiol is dominan in thefall leaf stress, while POD is dominan in ethephon treatment.



(A) (B)

Figure 1: Thiol content (mM) in health and TPD affected plant of the RRIM 912 and PB 260 latex (A= March and B = May).



(A) (B)

Figure 2: Thiol content (mM) in the bark of health and TPD partial plant in March and May. (A= March and B = May).

The content formulas shows the association of formulas in improving of stress tolerance plants through formulas supporting the performance of POD in the detoxification process with both enzymatic and non-enzymatic reactions. Although ethrel treatment increases physiological stress in rubber plants due to tissue injury which causes the sink and source process to be unbalance. By combining ethrel and 100 ppm and 150 ascorbic acid formulas as if to provide injury and healing at the same time so that even though they are still able to increase latex POD activity it is proven by lower control plants in March observations with the highest content below 0.3 units / ppm compared to ethrel and formula which reached more than 0.4 units / ppm. This is presumably because of the ascorbic acid content which acts as an antioxidant so that it can support the role of peroxidase in the process of removing ROS molecules such as H_2O_2. Winarsih (2000) mention the main target of ROS is protein, unsaturated fatty acids and lipoproteins, and DNA elements including carbohydrates and RNA. Unsaturated fatty acids are the most vulnerable to ROS attacks. Ardiansyah et al. (2014) said ascorbic acid functions as an antioxidant, cofactor enzyme and as a cell signal modulator in a variety of important physiological processes, including cell wall biosynthesis, secondary metabolites and phytohormones, stress tolerance, photoprotection, cell division and growth.

The control plants there was an increase in the content of POD which was very drastically observed in May compared to March, this is thought to be related to the deciduous process of leave fall session, where May is the beginning of re-formation of leaves so that the plants are no longer in stress. Gunasekara et al., (2013) said that there were differences in the production and response of each clone to the seasonal pattern. The results showed that PB260 clone activity looked more stable towards changes in seasonal patterns compared to RRIM 912 clones which showed a very drastic difference. This is in accordance with Siregar's statement (2016) PB 260 prioritizes thiol function in controlling stress while in low metabolic plants prioritizes POD enzymes. Siregar (2014) also said that canopy in PB260 is generally thicker and thicker concentrating on the number of fallers more and has a fast response to changes in rainfall. This is related to the hypersensitivity resistance gene that is dominantly expressed in plants, the gene encoding peroxidee enzyme and polyphenol peroxidase. Both of these enzymes more commonly play a role in defense mechanisms against diseases so that their activities are used as resistance induction (Saravanan et al., 2004).

Thiol contents in latex generally will increase if the plant is exposed by TPD and given stimulants, especially in PB260 plants. However, for healthy plants the content will decrease. This happens related to the level of exploitation and metabolism of each clone. Research by Sumarmadji (2000) says that the analysis of latex content in partial TPD plants is known to show low thiol content even though the plants were given stimulants ethepon and Tistama et. Al (2006) which stated that thiol and ATP in healthy plants were higher than those attacked by TPD. This is thought to be an attempt by the plant to overcome the conditions of oxidative stress stress. According to Anggraini (2016), PB260 is more susceptible to attack by TPD.

Higher thiol content in PB260 bark compared to RRIM 921 clones showed quick stater clones to experience higher exploitation than slow stater. This is an indication that slow stater plants are more tolerant of TPD. The higher thiol content of the bark can also indicate the presence of TPD whereas in healthy plants the content of thiol bark is lower even though it has been given stimulants up to 5% this is because the plants give a better response to stimulants combined with 100 ppm NAA formula and Ascorbic Acid 150 ppm (Figure 4). Siregar et al., (2008) reported that quick starter clones have a characteristic that is the peak pattern of latex production occurs in the early period, stimulant responsiveness, susceptible to TPD and thin skin recovery, while slow starter clones reach peak production in the middle of tapping period, stimulant response, relatively resistant to over-exploitation and thick skin recovery.

4 CONCLUSIONS

The Difference of antioxidant activities in the bark and latex rubber plants (*Hevea brasiliensis*) change according to environmental factors, clones, and physiological disorders in this case because of the provision of stimulants and nutrients and ascorbic acid. Increasing the value of thiol can reduce the value of protein and POD activity. Some parameters that can be used as early indicators for detecting TPD are bark POD activity and thiol latex value and based on observations in the field of using 100 ppm NAA formulas and Askorbit Acid 150 ppm can reduce the level of TPD in rubber plants. From the results of this study it is recommended to further investigate the metabolism of rubber plant bark and its formulas in a longer period of at least 6 months to 12 months.

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