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IMPROVEMENT OF PHYSIOLOGICAL CONDITION AND LATEX FLOW OF RUBBER CLONES AFFECTED BY TAPPING PANEL DRYNESS IN RESPONSE TO COMBINATION NAPHTHALENE ACETIC ACID, ASCORBIC ACID, AND NUTRITION TREATMENTS

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SUMMARY

The TPD incident is one of the factors causing rubber estates to lose their production. The objective of this research was to arrange formulation for recovering of tapping panel dryness (TPD) chases, especially on PB 260 and IRR 42 clones. The experiment was divided into two experiments, that were arranged into a split plot design with two treatment factors, first the main plots of rubber plant clones (PB 260 and IRR 42), and second different NAA concentrations in the step 1. The best combination of the first experiment was added with different concentrations of ascorbic acid in the research experiment II. The physiological variables observed were sucrose, inorganic phosphate, thiol, latex production, and biochemical variables were SOD, APX, and POD enzyme activities. The TPD-affected plant could produce latex under treatment of NAA and nutrient for six months NAA 10 ppm treatment significantly affected on Pi content and had positive effect on latex production. Treatment of ascorbic acid also increased production in rubber plants and affected on POD and SOD activities. There were differences respond between high metabolism clone PB 260 and low metabolism clone IRR 42 to combination of NAA, nutrient, and ascorbic acid treatment.

Keywords: anti-oxidance enzyme, *Hevea brasiliensis*, latex production, tapping panel dryness

INTRODUCTION

Tapping Panel Dryness (TPD) is a disorder condition in which rubber plant bark is not able to produce latex. The percentage of occurrence of TPD on rubber plants in large plantations was reported to reach 7.5-15% (Andrianto and Tistama, 2014). Impaired metabolic balance and nutrient content in latex and bark tissue were the causes of TPD (Jacob et al., 1989). This condition was triggered by the exploitation that exceeds the ability of clone productivity, suboptimal maintenance of plants and low quality of tapping (Andrianto and Tistama, 2014). There are two characteristics found in trees infected with TPD; firstly, latex flow reduction and early latex coagulation occurs on the tapping grooves; and secondly, there was a reduction in the number of laticifere (Putranto et al., 2015).

TPD was induced by disoreder in nutrient balance in the latex, that was caused by removal of some organic materials as well as macro and micro-elements for tapping (Andrianto & Tistama 2014). The main organic ingredients that were also extracted in latex were protein, sugar and carotene, while the macro and micro elements that were attached with the latex were N, P, K, Mg, Mn, Zn and others. The ability of the blood vessel tissue took 24 to 72 hours to extract them. Due to the intensive drainage, the ability of plants to regenerate latex, including protein and nutrients, was unbalanced. Such plant conditions are

called physiological fatigue (Jacob et al., 1989).

High tapping intensity induced TPD incident in 5 months and increased reach 75% after 24 months after opening (Herlinawati & Ismawanto 2017). Intensive tapping induced many functions disorder in latex cells and rise the production of reactive oxygen species (ROS) (Putranto et al. 2015). The accumulation of ROS was responsible for the internal latex coagulation in laticifer cells, caused a partial or complete stoppage of latex flow. Enzymatic and non-enzymatic antioxidants were the defense systems that were effective to suppress excessive ROS (Mitchel and Contran, 2008). Superoxide dismutase (SOD) was one of the important antioxidant enzymes which was derived from the body of rubber plant itself, has a very strong effect and was the body's first line of defense in dealing with oxidative stress (Rajkumar et al., 2008).

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, as well as cell division and growth (Ardiansyah et al., 2014). Thiols, ascorbate, and γ-tocotrienol are the major antioxidants in latex. They are involved in membrane protection from ROS and likely have an effect on the quality of raw rubber (Zhang et al., 2017). Exogenous ascorbic acid treatment increase rubber yield on TPD-affected trees (Satrio et al., 2016), that mean there are latex metabolism improving in laticifere cells.

Kumari and Nugawela (2013) reported that a lower tapping system treatment on TPD-affected trees show some progress in biochemical and physiological aspects, unfortunely the physiological process from healthy trees to those infected by TPD was still poorly understood. Renew bark has to accelerated to get healthy laticifer tissue. The physiological role of Naphthalene Acetic Acid (NAA) is to encourage cell elongation, differentiation of xylem and phloem tissues and root formation (Taiz and Zeiger, 2002). The highest content of auxin in the rubber tree bark was found in the age of 2 years is on rubber clones PB 260 (116.5 ppm) and RRIM 712, respectively 116,5 ppm and 114.0 ppm (Koryati and Siregar, 2013). In tissue culture, the addition of auxin serves to stimulate the growth of callus, and division cells as well as elongation of cells and organs, and it also stimulates apical dominance in meristem tissue (Takatsuka and Umeda, 2014). The combination of exogenous jasmonic acid with NAA affected on laticifer differentiation, and physiological in latex of *Hevea brasiliensis* (Tistama et al., 2017). The induction of the new healthy laticifer cells is one of approaching for recovery a normal latex productivity.

Physiological status in the rubber plant clones was specific depending on the type of metabolism and the TPD stage. Inorganic phosphate content and peroxidase activity were interesting to deeply investigate, particularly to observe its correlation with the TPD tolerance of the clones (Tistama et al., 2019). It was previously reported that the thiol contain in PB 260 (high metabolism clone) were significantly lower in the TPD-affected trees compared to the healthy trees (Putranto et al., 2015). The type of rubber clones and different plant age have different content auxin in bark (Koryati and Siregar, 2013). Based on the reports, it is considered necessary to conduct research in the differents concentration of NAA, ascorbic acid, and nutrition on TPD-affected plants. For the most part, research on TPD have been focusing on latex analysis. This research aimed observing NAA, ascorbic acid, and nutrition treatments on the physiological status correlated with the stage of TPD in two types of rubber plant metabolism in latex and bark.

MATERIALS AND METHODS

This research was conducted in two-step trials. The first step was an experiment to found an optimal combination of different concentrations of Murashige and Skoog media and auxin (NAA) effect on the recovery of TPD-affected plants. In the second step,

the optimal combination in the first experiment was enriched with different concentrations of ascorbic acid. The research was conducted in the rubber plantation of Sungei Putih Research Centre, Deli Serdang Regency, North Sumatra. The two types of clones, PB 260 which has high metabolism of latex and IRR 42 which has low metabolism of latex were selected in this experiment. The two rubber clones were planted in 2006 to 2007 at ± 54 m above sea level and type B climate based on the Oldeman classification (7-9 consecutive wet months). The plants were planted with a spacing of 6 x 3 m and used a taping system of 1/2S ↑ d3ET.2.5%.6/y (m). Other tools and materials needed in this research include chemicals for latex diagnosis, chemicals for viewing the histology of the network of latex vessels, marker markers, oil paints, tapping knives, spectrophotometers, and light microscopes.

Plants selected as sample plants were plants with complete TPD infection (75-100%) (Experiment I) and plants with partial TPD infection (50-75%) (Experiment II). The sample plants criteria were also selected on the girth homogeneity, canopy, and white root fungus disease free. Each treatment consisted of 5 plants and was replicated 2 times. The observations were conducted for 2 months after 4 months of treatment (4AT). The results of the research demonstrated a significant effect would be tested by the Tukey distance test at 5% confidence level (Steel and Torrie, 1995).

Experiment I

The first experiment of research applied Split Plot design. The main factors were different concentration of NAA were 0 ppm (N0), 10 ppm (N1), 20 ppm (N2), 30 ppm (N3), and nutrition referred to Murashige and Skoog (MS) composition (Murashige and Skoog, 1962). The nutrition were consisted two treatments with (one concentration of MS composition) (V0) and without MS treatment (V1).

Experiments II

The second experiment of research employed a split-plot design consisting of treatments. The main plots were clones (PB 260 and IRR 42) and subplot different concentrations of ascorbic acid consisted four level were 0 ppm, 50 ppm, 100 ppm, and 150 ppm.

Procedure

Candidates of sample plants were clearly marked with paint or tied by ropes to avoid observation errors and the sample plants were tapped by special tappers with total of 48 samples and 3 repetitions. Application of hormones and nutrients on the tapping area carried out by bark scrapping with a knife to remove the outer bark, approximately 1-2 mm thick. NAA and nutrients were mixed with glycerin and sprayed on the tapping areas of rubber three which bark had been scrapped 1 mm. The interval for treatments was once a week for 6 months. The parameters observed were similar to those in the first research step, namely analysis of thiols, sucrose, Pi, peroxidase, SOD enzymes, and latex production.

Protein Extraction and Peroxidase Activity

Protein extractions were performed following procedures proposed by Packeer-Mohamad et al. (2012). The soft tissue of barks (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 centrifuged at 12.000 rpm, 4°C for 15 min. The supernatant was separated for protein quantification and analysis of peroxidase activity. Protein contents were measured by Bradford methods using BSA as standard (Bradford, 1976). One hundred microliters of protein extraction samples were added with 100 µl 1M NaOH to each sample and vortex. Protein standard was prepared with containing a range of 5 to 100 microgram of protein (albumin). Each mixture was added with 5 ml dye reagent and incubated 5 minutes, and measured the absorbance at 595 nm. Peroxidase activities were analyzed according to the method proposed by Shannon et al. (1996). The reaction mixture

consisted 2.775 mL of 0.05 M sodium acetate buffer, pH 5.4, 100 µl of 0.25% (w/v) o-dianisidine, 100 µl of 0.1 M H_2O_2, and 25 µl of enzyme solution. Absorbance at 460 nm was recorded every 15 second for 2 min.

Data Analysis

The effect of TPD stage in each clone was tested using ANOVA and Tukey Multiple Comparison at alpha = 5%. Correlation between parameters was tested by Pearson Correlation analysis.

RESULT AND DISCUSSION

Aplication of NAA and Nutrition on the Bark

Generaly, NAA and nutrition treatment affected on latex production after 6th month application, both in PB 260 and IRR 42. The effect was significantly only on the single treatment, but the combination NAA-nutrition treatments were not significantly affected on the production increasing. NAA may induced cambial activity and laticifer differentiation. Koryati and Tistama (2020) reported that paclobutrazol application on the bark increase trunk girth and bark thickeness and enlargement laticifer cells. NAA induce cambial activity in wild type of Arabidopsis (Suer et al., 2011). Laticifere differentiation was affected by exogenous NAA combined with jasmonic acid (Tistama et al., 2017).

The Murashige and Skoog (MS) composition solution was effective to increase latex production in rubber plants. Exogenously macro and micro elements might either increase the nutrition or made nutrition balance in the bark of TPD-affected

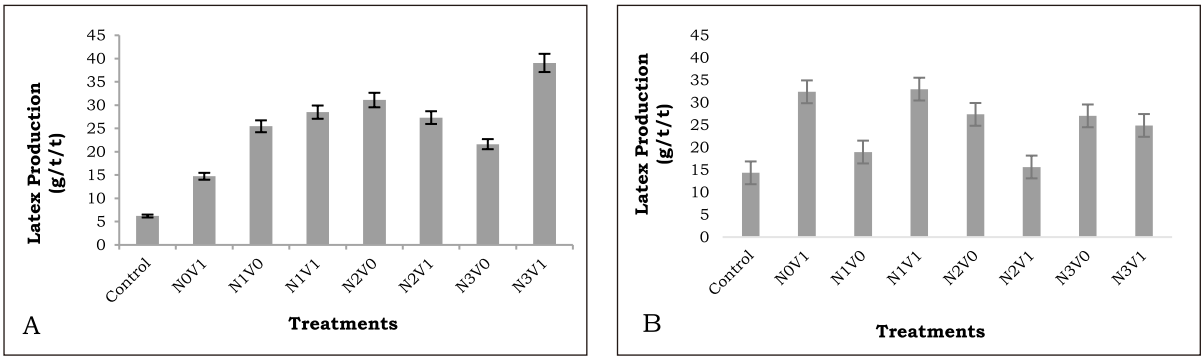

Figure 1. Effected of NAA and nutrition on latex production gram/tree/tapping (g/t/t) in PB 260 (A) and IRR 42 (B), 6st month after application.

Table 1. Effected of NAA and nutrition on latex production gram/tree/tapping (g/t/t) in PB 260, 6th month after application. NAA concentrations were 0 ppm (N0), 10 ppm (N1), 20 ppm (N2), 30 ppm (N3)

Treatment MS	Nutrition				Average
	N0	N1	N2	N3	
V0 (Without)	4.30	18.96	27.30	27.03	19.40b **
V1 (with)	32.40	33.00	15.63	24.90	**26.48a** **
Average	18.35c	**25.98a**	21.47b	25.97a	

Remark: different letters in each column indicated the significantly difference (p<0.05) by LSD multiple range test. All data were presented as mean calculated from three independent replicates

plants. The nutrition sufficiency in the bark might induce normally latex metabolism and regenerate the new healthy cell form. Physiological parameters in the latex, such as sucrose, thiols, and inorganic phosphorus contents, were slightly induced by nutrient sufficiency and this positive physiological change might related to yield increase after fertilization (Tiva et al., 2016). The regeneration bark cells related with cambial activity. Besides hormone role, the nutrient also affected to cambial activity including the cells differentiation. An article reported that N, P, K, and Mg deficiency in reduce phloem thickness, cell size, as well as number and size of latex vessel tissue (de Fay and Jacob, 1989).

In this experiment, the nutrient status in the latex and bark were not measured, so there were not data that supported relation between the changes of nutrient status to latex production. According to Joseph (2006), the contents of

Table 2. Effected of NAA and Nutrition on latex production gram/tree/tapping (g/t/t) in IRR 42, 6th month after application. NAA concentrations were 0 ppm (N0), 10 ppm (N1), 20 ppm (N2), 30 ppm (N3)

Treatment MS	Nutrition				Average
	N0	N1	N2	N3	
V0 (Without)	4.30	18.96	27.30	27.03	19.40b **
V1 (with)	32.40	33.00	15.63	24.90	**26.48a** **
Average	18.35c	**25.98a**	21.47b	25.97a	

Remark: Different letters in each columns indicated the significantly difference ($p<0.05$) by LSD multiple range test. All data were presented as mean calculated from three independent replicates.

nutrients P, Mg, and Mn were higher, whereas Fe and Zn were lower in latex of TPD-infected plants compared to normal plants, while the concentrations of P and K were higher. The micro elements were important role to maintain membrane stability and plant metabolism (Leiwakasbessy et al., 2003). Mg deficiency in TPD-infected plants could increase the instability of latex vessels (Sivakumaran et al., 2002).

One of the main factors on latex metabolism is phosphate inorganic as energy source in this pathway. The application of NAA had insignificant effects on Pi at the 6th-month observation, while the metabolism and nutrition had an insignificant effect.

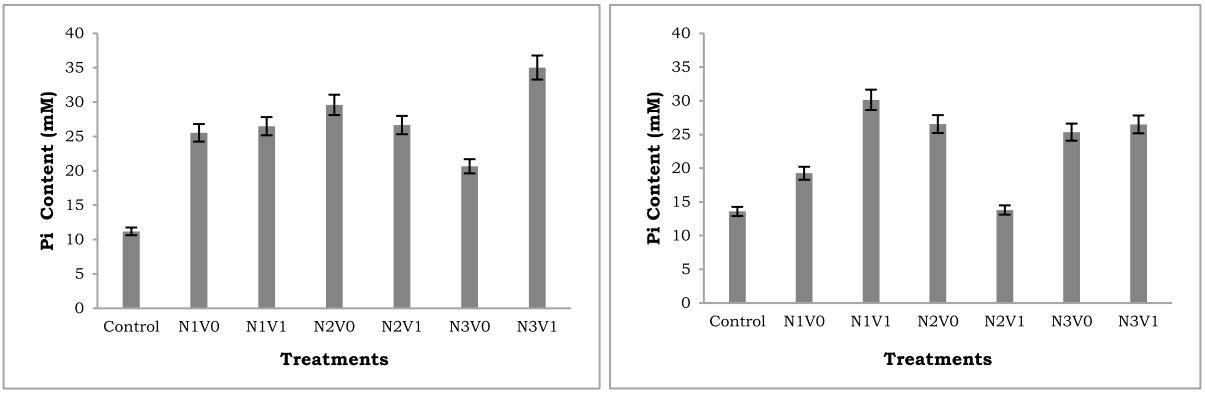

Figure 2. The different concentrations of NAA Hormones and nutrition affected on latex phosphate inorganic (Pi) latex TPD-affected tree, clone PB 260 and IRR 42. Note: N1, N2 and N3 were NAA contain respectively 10, 20, and 30 ppm; V0 was without nutrition and V1 was with MS nutrition

Metabolic interactions, NAA administration, and nutrition also had insignificant effects (Figure 2). Pi content in the plant treated by NAA nutrition and the control plant were categorized in medium (12 - 15 ppm), that mean the latex metabolism were normally running.

Effect of Exogenus Ascorbic Acid

The treatment of ascorbic acid affected on rubber yield especially in high metabolism clone PB 260 which had a significant increase at the concentration of 50 ppm; while for IRR 42 clones had only a slight increase. Rubber yield reached 50% in IRR 42 on 100 ppm concentration treatment. The fact show that the low metabolism clone IRR 42 required higher concentration of ascorbic acid to induce those of rubber yield than high metabolisme clone PB 260 (Fig. 3). Satrio et al. (2016) reported that ascorbic acid application significantly increase latex production, however not significant affected on the latex physiology parameters.

POD activity of PB 260 in control was 10 times lower than the activity in IRR 42. The ascorbic acid treatment induced POD activity in PB 260 5-7 times than control, while in IRR 42, the treatments tend to inhibited POD activity. The highest POD activity in PB 260 was found in the treatment of 50 ppm and the activities decreased by the increasing of ascorbic acid concentration. In

Table 3. Effects of ascorbic acid application on latex production gram/three/tapping (g/t/t) of partial TPD–affected tree, clone PB 260 and IRR 42.

Treatment Clone	Ascorbic acid Concentration				Average
	Control	50 ppm	100 ppm	150 ppm	
PB 260	9.67	27.36	17.16	19.13	**18.33a** **
IRR 42	5.37	13.46	21.37	17.06	14.32b **
Average	7.52c	**20.41a**	19.27a	18.10b	

Remark : fifferent letters in each column indicated the significantly difference ($p<0.05$) by LSD multiple range test. All data were presented as mean calculated from three independent replicates.

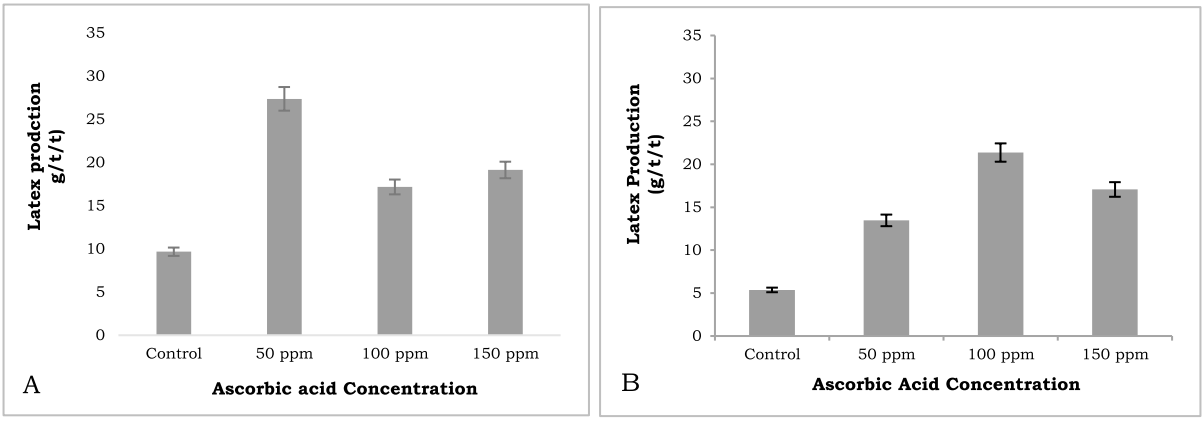

Figure 3. Effects of ascorbic acid application on latex production gram/three/tapping (g/t/t) of partial TPD–affected tree, clone PB 260 (A) and IRR 42 (B)

latex IRR 42, the ascorbic acid repressed the POD activity on 50 and 100 ppm concentrations, however the activity turn increased on 150 ppm ascorbic acid treatment (Fig 4).

Salama et al. (2014) stated that ascorbic acid was a natural product from plants that had an important function as an antioxidant and enzyme and seemed to have an important role to reduce cofactors. Ascorbic acid also can inhibit reactive oxygen

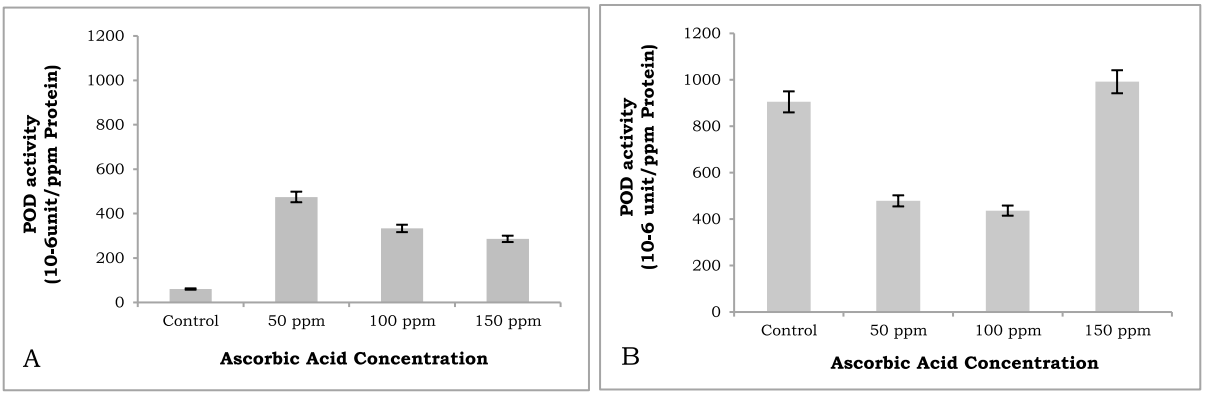

Figure 4. Effect of different concentration of ascorbic acid treatment on POD activities in latex of TPD-affected trees, clone PB 260 (A) and IRR 42 (B)

species (ROS) (Fukumura et al., 2012). Ardiansyah (2014) reported that one of the efforts to increase tolerance to oxidative stress was the application of ascorbic acid. Foliar-applied ascorbic acid caused a marked improvement in shoot and root fresh and dry weights, plant height, chlorophyll, and the activity of peroxidase (POD) enzyme particularly under water deficit conditions (Farooq et al., 2020).

SOD activity under different concentrations of ascorbic acid treatment had a difference pattern in PB 260 and IRR 42. In PB 260, SOD increased higher than the control in low concentration of ascorbic

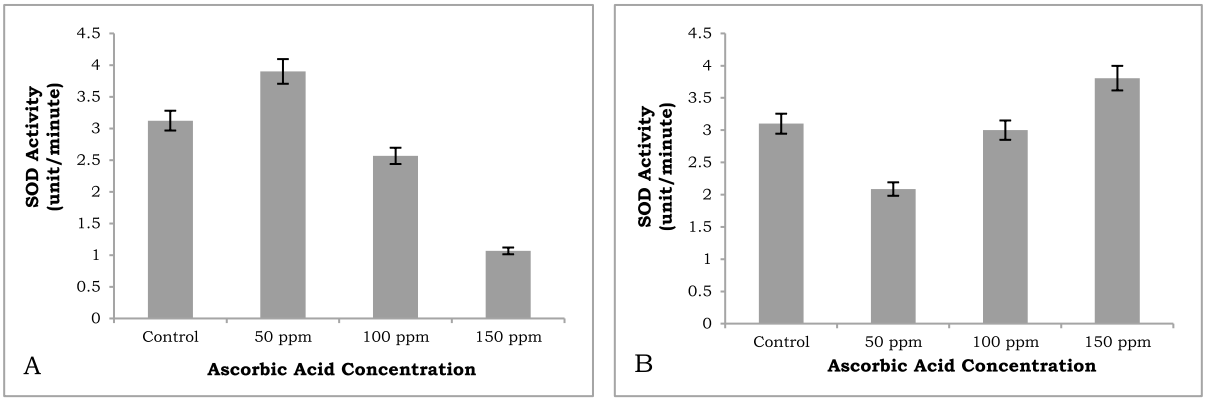

Figure 5. The change of SOD activities due to exogenously different of ascorbic Acid concentrations on TPD-affected trees, PB 260 (a) and IRR 42 (b).

acid treatment and then decreased in higher of it concentration. The pattern of SOD activity in IRR 42 was low in low ascorbic acid treatment, and increased along with higher concentrations of ascorbic acid (Fig 5). Exogenously applied Ascorbic acid lowered some stress substances indicator as the contents of MDA and H_2O_2, and the activities of CAT and SOD enzymes (Farooq et al., 2020).

Correlation analysis of some oxidative enzymes shows that only SOD value had positive correlation to latex productivity 0.59, whereas APX and POD had a negative correlation to latex production respectively -0.39 and -0.46. The increase in SOD activity was affected by the protein content and POD values (Table 4). SOD and POD activities could be latex production indicators and demonstrate physiological status of rubber plant metabolism.

Ascorbic acid was the first substance to detoxify and neutralize superoxide radicals. Ascorbic acid also had an important role in photo-protection, photosynthesis regulation, and plant growth processes such as cell division and cell wall expansion (Taiz and Zeiger, 2003). The exogenous ascorbic acid application was also known to increase latex production and also reduced tapping panel dryness (TPD) in commercial rubber plantations (Satrio et al., 2016). Each rubber clone has specific productivity responses to exogenous ascorbic acid depending on metabolism character. However, the ascorbic acid treatment showed a higher increase latex production than those of in control plant group. Thus, it can be explained that the application of ascorbic acid had a positive effect to latex production.

Table 4. Correlation of some oxidative enzymes toward latex production under ascorbic acid treatment in PB 260 and IRR 42

Parameter	SOD	APX	POD
SOD	1		
APX	-0.08	1	
POD	-0.3	-0.55	1
Protein	-0.76	-0.1	-0.18
Production	0.59	-0.38	-0.46

CONCLUSION

The treatment of NAA and nutrients had a positive affected on Pi content and was able to induce latex production in the complete TPD-affected plant. The treatment of ascorbic acid also increases latex production of TPD-affected plant and it role in the anti-oxidance enzyme activity. PB 260 differed from IRR 42 in responding to exogenous NAA and ascorbic acid both changed in physiological status and latex production.

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