

ACTIVITIES OF ASCORBIC PEROXIDASE (Apx) And TANNIN CLONES PB 260 ON TAPPING PANEL DRYNESS (TPD)

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ABSTRACT

TPD was a physiological caused by overstimulation ethylene an overproduction of reactive oxygen species (ROS). As an adaptive response, plant produce antioxidant such as secondary metabolites one of them was ascorbic acid (AsA). Exogenous application of AsA has been very effective in improving plant growth and development by altering oxidative defense system and induction of tannin synthesis in response to environmental stresses may also indicate potential functions. These Research aimed to provided activity of ascorbic peroxidase, tannin and production in *Hevea brasiliensis* Clones PB 260 affected Tapping Panel Dryness and to find the effect of ascorbic acid on production latex in in *Hevea brasiliensis* Clones PB.

The research was conducted in rubber plantation of Sungei Putih Research Institute, Deli Serdang Regency North Sumatra with PB 260 as clone were planted in 2006 to 2007 at ± 54 m above sea level. The interval for treatment was once a week for 4 months. The observation was made 4th months after first application. The parameters observed were production, the activity of Ascorbic peroxidase and tanin. The result showed Applied ascorbic acid 150 ppm can increased the production of latex and tannins in rubber plants that are exposed to tapping panel dryness (TPD) but reduced the activity of the ascorbate peroxidase (Apx).

Keywords: Apx, Ascorbic acid, Tannin, PB 260

INTRODUCTION

The Tapping Panel Dryness (TPD) syndrome is characterized by the partial or, ultimately, total stoppage of latex flow upon tapping, due to *in situ* coagulation of rubber particles. Numerous studies have shown that TPD appears to be a physiological disorder resulting from excessive recurrent tapping and overstimulation by ethylene (Obouayeba, 2011). This form of TPD is related to an overproduction of reactive oxygen species (ROS) in laticifers, called ROS-TPD in this manuscript. 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 signalling genes. The predicted function for some Ethylene Response Factor genes suggested that these candidate genes should play an important role in regulating susceptibility to TPD (Putranto et al., 2015).

The deficiency at any stage of plant growth and development can change in metabolism, physiology and morphology (Ashraf *et al.*, 2011) due to accured of Reactive Oxygen System (ROS). Generally, under stress conditions, plant hormonal signaling results induction of various acclimation responses, ultimately regulating plant physio-biochemical processes (Mittova *et al.*, 2015). Hormonal and ROS signaling networks are highly integrated and both collectively induce appropriate responses under stress by processing and transmitting environmental inputs (Baxter *et al.*, 2014).

As an adaptive response, plant produce antioxidant such as secondary metabolites (Halimeh *et al.*, 2013). These ROS scavengers in the form of enzymatic or non-enzymatic antioxidants such as POD, CAT, SOD, tocopherol, ascorbic acid, glutathione and phenolics, etc. (Weng *et al.*, 2015). Physiologically active form of AsA is the resonance stabilized anionic form which is termed as ascorbate. Likewise, ascorbate anion (AsA^-) is a vital water-soluble antioxidant molecule within biological system (Smirnoff, 2000).

Ascorbic acid (AsA) is a non-enzymatic complex which enables plants to defend against stresses by reducing oxygen free radicals (Shafiq *et al.*, 2014). AsA is generally distributed in the cytosol of the plant and it acts as an antioxidant. Moreover, the plants with low ascorbate synthesis are quite sensitive to different environmental stresses which can adversely affect their growth and development (Ahmad *et al.*, 2013). Pignocchi *et al.* (2006) reported that redox states of the apoplastic ascorbate levels influence hormonal balance, growth responses, MAPK signaling cascades and antioxidant enzyme activities, while glutathione levels remain unaffected. Consequently, due to its apoplastic localization, AsA constitutes a vital role in stress perception, redox homeostasis and subsequent regulation of oxidative stress and plant physio-biochemical responses under normal as well as different abiotic stresses. Exogenous application of AsA has been very effective in improving plant growth and development by altering oxidative defense system, phytohormone signaling, cell expansion, ion transports and other related processes under stress or non-stress conditions (Darvishan *et al.*, 2013).

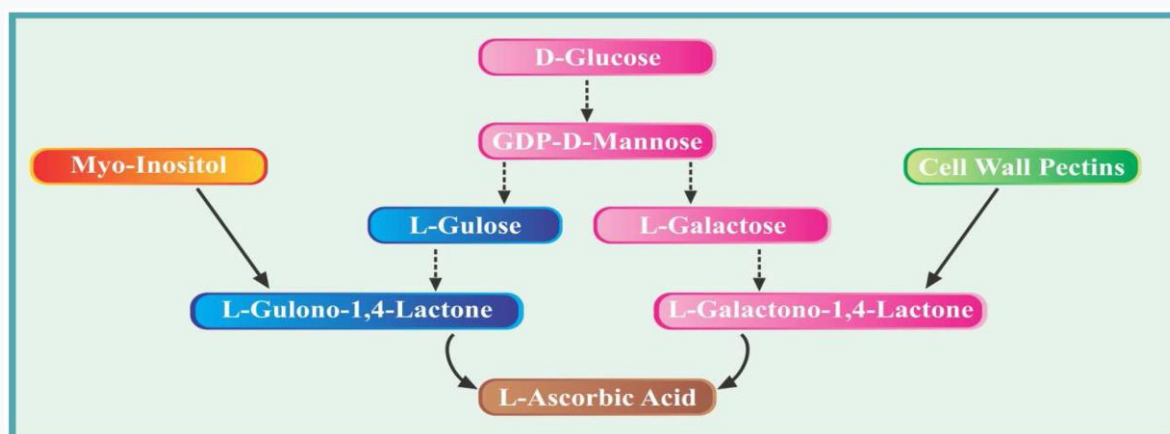


Figure 1. Diagram showing various pathways and important precursor molecules involved in biosynthesis of ascorbic acid in plants (Akram *et al.*, 2017).

Tannins are chemically diverse, but are functionally defined by their ability to bind protein. Although much research has been driven by early work postulating that the protein-binding ability of tannins is important in defense against insect pests, currently the best

evidence for the antiherbivore role of tannins comes from work with mammals. The role of tannins in defense against insects and mammals has been reviewed recently (Barbehenn & Constabel, 2011). Tannins can have pronounced effects on nutrient cycling and can affect entire ecosystems. At the scale of the individual plant, it is becoming clear that tannin synthesis is precisely regulated and is often restricted to specific cells or tissues. Therefore, knowledge about a tannin's localization will provide clues as to its function in the plant. Similarly, the induction of tannin synthesis in response to environmental stresses may also indicate potential functions (Constabel et al., 2014). 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).

This research purposes is to provide activity of ascorbic peroxidase in *Hevea rasiliensis* Clones PB 260 affected Tapping Panel Dryness and to find the effect of ascorbic acid on production latex in in *Hevea brasiliensis* Clones PB

RESEARCH METHODS

The research was conducted in rubber plantation of Sungei Putih Research Institute, Deli Serdang Regency North Sumatra. The types of clones, PB 260 which has high metabolism of latex were planted in 2006 to 2007 at ± 54 m above sea level and type B climate based on Oldeman classification (7-9 consecutive wet months). The plant material used was the group of plants that had been selected planted in 2005-2006 with a spacing of 6 x 3 m with a tapping system of 1/2S $\uparrow$ 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. The sample plants criteria was also selected on the girth homogeneity, canopy, and white Root fungus disease free. Each treatment was consisted 5 plants and replicated 2 times. The observations were conducted for 2 months after 4 months of treatment (4AT).

Research Implementation

The initial step of research was plotting of the area. Candidate of sample plants were clearly marked with paint or tied by ropes to avoid observation errors and so that the plants which were used as sample plants were not tapped by tappers. After obtaining sample plants with complete TPD infection, then, before application of hormones and nutrients in the tapping area that would be treated, the barks were firstly scraped (bark scrapping) with a knife to remove the outer bark for approximately 1-2 mm.

Ascorbic acid 150 ppm and nutrients was mixed with glycerin and sprayed on the tapping areas of rubber three which bark had been scrapped 1 mm. The interval for treatment was once a week for 4 months. The observation was made 4th months after first application.

The parameters observed were production, the activity of Ascorbic peroxidase and tanin. The method of Moons et al. (1997) was followed to analyze the ascorbate peroxide (APX, EC 1.11.1.11) activity. The reaction solution contains 100 mM phosphate (pH 7.0), 0.3 mM ascorbic acid, 0.06 mM H₂O₂, 0.1 mM EDTA-Na₂ and 100 μ l enzyme extract. The spectrophotometer was set at 290 nm and the absorption was taken 30 s after addition of H₂O₂. For tanin analysis was 5 grams of rubber bark finely ground and add 400 ml of distilled water then boil for 30 minutes, then put it in a 500 ml measuring flask and add distilled water to the mark, then filtered (filtrate I). 10 ml of filtrate I was taken plus 25 ml of indigocarmine indicator and 750 ml of aquadest. Then titrated with 0.1 N KMnO₄ solution

until golden yellow color, for example A ml is needed. Take 100 ml of filtrate I plus 50 ml of gelatin solution, 100 ml of acid salt solution, and 10 grams of kaolin powder, respectively. Then shaken vigorously for a few minutes and filtered (filtrate II). Taken 25 ml and aquadest 750 ml then titrate with 0.1 N KMnO₄ solution, for example required B ml. Standardization of the KMnO₄ solution with Na-oxalate. 1 ml KMnO₄ 0.1 N = 0.00416 gr tannins. The results of research demonstrated a significant effect would be tested by Tukey distance test at 5% confidence level (Steel and Torrie, 1995).

RESULTS AND DISCUSSION

Result

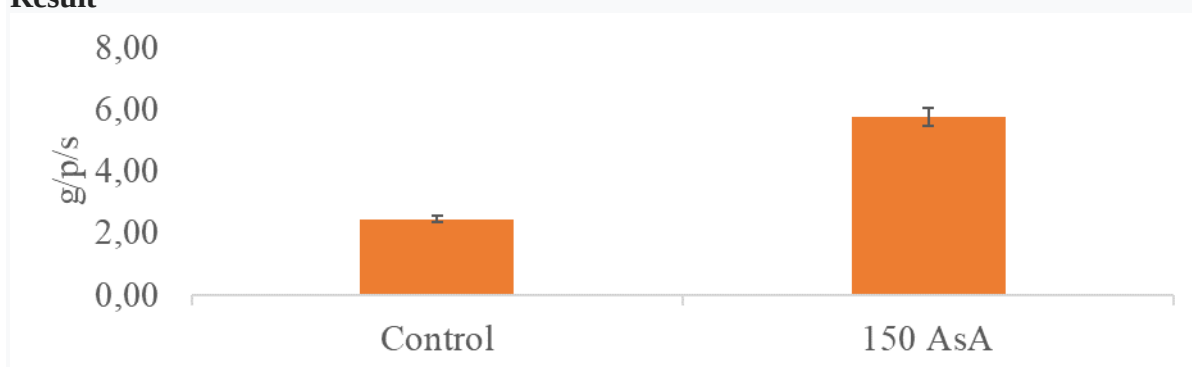


Figure 2. Effect of applicant acorbic acid to latex production on TPD condition

Application of nutrient solution and 150 ppm ascorbic acid increased the production of PB 260 clones by about 2 times more than control plant. Even though 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 (Tistama et al., 2019). The results of the analysis are presented in Figure 2, AsA can improve plant growth and yield as a potential regulator of different mechanisms under adverse factors, so over-accumulation of AsA in plants through gene (s) engineering could efficiently increase plant stress tolerance. Yet, not a single report in the literature is available on stress tolerant genetically modified plants with high accumulation of AsA. Overall, high accumulation of AsA in plants under stress conditions is linked to stress tolerance mediated by AsA scavenging ROS and upgrading the oxidative defense potential of the plants, resulting in better growth and development under stress conditions (Akram et al, 2017).

The continued latex production when partial TPD occurred might be explained by the large latex-producing zone of the tapping panel involved in latex flow and only local oxidative stress in part of the tapping cut. Latex diagnosis is an efficient method for early detection of laticifer dysfunctioning. Typically, an increase in sucrose content and a decrease in thiols content were observed. When TPD symptoms occurred significantly, all the latex diagnosis parameters fell slightly but significantly, revealing a general decrease in metabolic activity in the laticifers (Putranto et al., 2015).

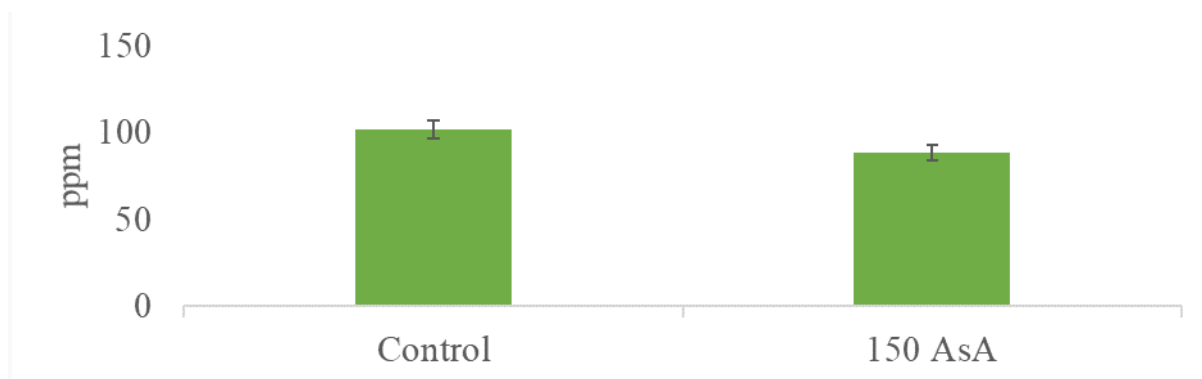


Figure 3. Effect of applicant acorbic acid to Ascorbic peroxidase on TPD

Ascorbic acid (AsA) is known to protect organelles and cells from the adversaries of ROS, which over-accumulate due to stress-induced oxidative damages (Latif et al., 2016; Mukhtaret al., 2016; Naz et al., 2016). It controls cell division and cell expansion, acts as a cofactor of many enzymes, modulates plant sense, and is involved in photosynthesis, hormone biosynthesis, and regeneration of antioxidants (Gallie, 2012; Lisko et al., 2014). Although AsA is found in different plant tissues, its levels are abundant in meristems, photosynthetic cells and different fruits (Hong Bo et al., 2005; Mazid et al., 2011). Higher concentration of AsA has been reported in fully developed chloroplasts of mature leaves. Under normal conditions, AsA is available mostly in reduced form (Khan et al., 2011).

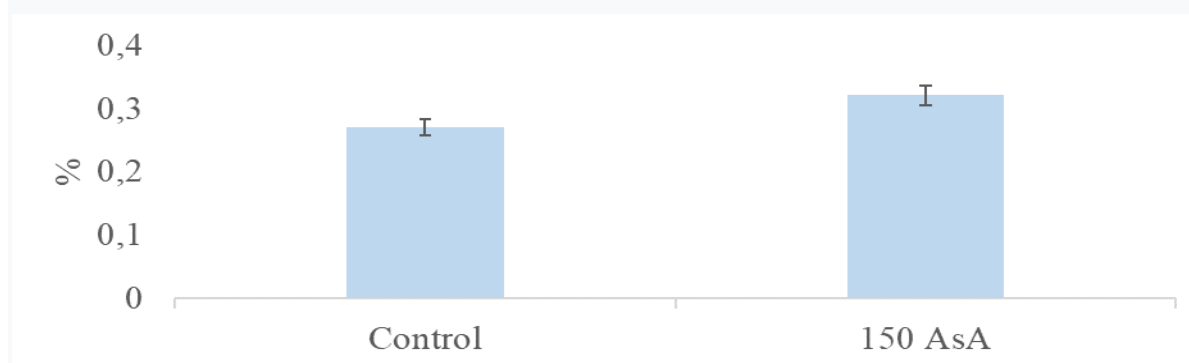


Figure 4. Effect of applicant acorbic acid to tannin on TPD condition

In plants, isoprene is a dual purpose metabolite that can act as thermo-protective agent proposed to prevent degradation of photosynthetic enzymes/membrane structures and as reactive molecule reducing abiotic oxidative stress. A transcriptional down-regulation of genes encoding enzymes of phenylpropanoid biosynthetic and regulatory pathways, as well as distinct metabolic down-regulation of condensed tannins (Duque et al, 2013). the ability of tannins to bind proteins, to act as antioxidants or pro-oxidants, and to chelate iron and other metals (Constabel et al., 2014).

Condensed tannins are a group of water soluble phenolic compounds with molecular weight ranging between 0.5-3.0 KDa. Positive correlation between total phenolics, condensed tannins and antioxidant properties were observed by various workers (Zhiping et al., 2011; Kunyanga et al., 2011). Though other antioxidants were probably present in these crops, the phenolic compounds could make a significant contribution to the antioxidant activities (Pal et al., 2013).

CONCLUSIONS

Plants have some substances can be found in structure of them which they have essential role called plants secondary metabolites to responses abiotic stresses. deficiency can change metabolism, physiology and morphology. In *Hevea brasiliensis*, application of ascorbic acid 150 ppm can increase the production of latex and tannins was exposed to dry tapping dryness (TPD) but reduced the activity of the ascorbate peroxidase enzyme (Apx).

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