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Research Article
Trypsin Inhibitor Activity and Protein Analysis of Gall Rust from Sengon Plants (*Falcataria moluccana* Miq.) Infected with *Uromycladium tepperianum* Fungus

Alfi Rumidatul, Endah Sulistyawati and I Nyoman Pugeg Aryantha

School of Life Sciences and Technology, Institut Teknologi Bandung, Jl. Ganesha No. 10, 40132 Bandung, West Java, Indonesia

Abstract

Background and Objectives: Sengon *Falcataria moluccana* (*F. moluccana*) has the ability to synthesize protease inhibitors, such as trypsin inhibitors, that can inhibit the activity of an enzyme produced by *Uromycladium tepperianum* (*U. tepperianum*), a fungus that causes gall rust disease. The purpose of this research is to study the activity of the trypsin inhibitor, the total protein content and the protein profile in healthy and gall rust infected sengon. **Materials and Methods:** The samples used in this research were taken from sengon twigs consisting of healthy and gall rust infected bark and wood. In this research, three age classes of sengon were used: 1-2 years, 3-4 years and 5-6 years. The method used in this research was field sampling, trypsin inhibitor activity analysis, protein determination and protein profile analysis by SDS-PAGE (Sodium dodecyl sulfate-polyacrylamide gel electrophoresis). **Results:** The results showed that, in general the activity of trypsin inhibitors in the gall rust infected sengon twigs was higher than in the healthy sengon twigs. The highest protein content was found in gall rust infected bark twigs and the lowest protein content was in healthy wood twigs. The results of the SDS-PAGE analysis of healthy and gall rust infected bark showed that there was a protein with a molecular weight of 21 kDa, which was identified as a trypsin inhibitor. **Conclusion:** *Falcataria moluccana* infected with *U. tepperianum* fungus showed an increase in the activity of trypsin inhibitors and protein content.

Key words: Trypsin inhibitors, sengon, gall rust disease, protein, SDS-PAGE

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Corresponding Author: Alfi Rumidatul, School of Life Sciences and Technology, Institut Teknologi Bandung, Jl. Ganesha No. 10, 40132 Bandung, West Java, Indonesia Tel: +62-81221508295

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Falcataria moluccana (Miq.) is one of the multipurpose pioneer tree species widely used in reforestation programs and the development of community forests in Indonesia. This species is also commonly planted in industrial forest plantations in Indonesia due to its rapid growth (fast growing species), adaptability to various soil types, good silvicultural characteristics and acceptable wood quality for panels and carpentry industries[1].

Gall rust, a type of tree disease caused by *Uromycladium tepperianum* fungus, has been widely reported to infect sengon trees. Gall rust has a distinct symptom, namely hyperplasia, i.e., excessive growth in the affected plant tissue. The symptoms of the disease start with local swelling (tumefaction) in the affected part of the tree and over time it will turn into lumps and then become small nodules called gall[2].

In general, plants have defense mechanisms to counter various types of unfavorable conditions including insects and phytopathogenic microorganisms. Plant's defense mechanisms consist of structural and biochemical defenses. The most important component of all defense mechanisms is the protein compound[3]. Research on the expression of protein against biotic environmental stress has been widely investigated. For example, Moshe *et al.*[4] reported that tomato plants express a defensive protein against TYLCV (tomato yellow leaf curl virus) infection.

Plant proteins that function as a defense against pathogenic infections are known as pathogenesis-related proteins (PR proteins). This protein is a specific protein present in plants that sustain plant life, especially in counteracting attacks from micro-organisms and viruses[5].

According to their properties and functions, PR proteins can be classified into 17 families. These proteins have molecular weights ranging from 6-43 kDa. One group of PR proteins is the protease inhibitors. Plant protease inhibitors are the plant's self-defense mechanism against predators or pathogens[6]. One example of a protease inhibitor is a trypsin inhibitor. Lopes *et al.*[7] stated that trypsin inhibitors play an important role in the mechanisms of plant defense against insects, fungus and other micro-organisms.

Trypsin inhibitors are widely found in plants and act as a plant defense strategy against pests and pathogens[8-10]. Research conducted by Siregar *et al.*[11] showed that the trypsin inhibitor in the sengon tree, especially in the bark tissue, is the Sengon's natural defense mechanism against the boktor pest. In addition to its role as a plant defense mechanism, trypsin inhibitors could also be used in various applications such as medicine, agriculture and food technology[12]. From a health perspective, many studies report that trypsin inhibitors are used for inhibiting carcinogenesis[13-15] and lowering blood sugar[16].

The defense mechanism of the Sengon tree against the boktor pest has been studied[11], however, there has been no research on the defense mechanism of sengon trees against gall rust disease caused by *U. tepperianum* or specifically on their trypsin inhibitor properties. Therefore, research on the properties of sengon trypsin inhibitors infected with gall rust disease is needed. The current research aimed to investigate the activity of the trypsin inhibitor, the total protein content and the protein profile in healthy and gall rust infected sengon trees.

MATERIALS AND METHODS

Field sampling: The plant material used in this research originated from *F. moluccana* or sengon stands grown in Cileles village, Jatinangor, Sumedang, West Java. The samples consisted of healthy twigs (bark and wood) and gall rust infected twigs (bark and wood). The plant samples were taken from trees of three different age classes: 1-2 years, 3-4 years and 5-6 years old. For each age class, three samples from different trees were collected for replication. The research for this study was conducted over 7 months from March-October, 2017.

Trypsin inhibitor activity analysis: The determination of trypsin inhibitor activity was carried out according to a study by Wong *et al.*[17]. The powdered sample as much as 1 g, was extracted with 10 mM of sodium hydroxide (NaOH) 50 mL for 3 h and the pH was adjusted to 9.4-9.6. The extract obtained from this procedure was diluted. Therefore, the percentage of supernatant inhibition defined as a 1 mL extract that will yield 40-60% inhibitory activity was reached. The diluted extract was treated with 5 mL of benzoyl-DL-arginine-p-nitroanilide (BAPNA), incubated in a water bath at 37°C for 10 min and then 1 mL of 30% acetic acid was added to it. The supernatant was measured by a spectrophotometer at a wavelength of 410 nm (UV-16001, Shimadzu, Japan). Trypsin inhibitor activity (TIA) is defined as the number of trypsin units inhibited. All the chemicals used for trypsin inhibitor activity analysis were purchased from Sigma-Aldrich (USA) specification were procured from local chemical vendor.

Protein extraction: Samples of bark and stem from healthy and gall rust infected sengon twigs as much as 4 mg, were immersed in 20 mL of distilled water for 24 h and then ground using a pestle and mortar until homogenous so that the extract or homogenate was produced. The homogenate materials were then macerated for 16 h at 4°C and then filtered using a filter cloth and subsequently centrifuged (5417 C; Eppendorf AG, Hamburg, Germany) at 12.000 rpm for 20 min at 4°C. The supernatant obtained by centrifugation was stored at -20°C for further use[18]. All the chemicals used for protein extraction activity analysis were purchased from Sigma-Aldrich.

Protein content determination: Determination of protein content was performed using the Lowry method[19]. Samples in the form of a supernatant from protein extraction were diluted with distilled water to 1.6 mL volume, with 600 µL of Lowry C reagent added. This was then vortexed and allowed to stand for 10 min. About 200 µL of Lowry D reagent was added to the supernatant and allowed to stand for 30 min. This mixture was measured at absorbance 750 nm in a spectrophotometer against blank samples. Protein concentrations were determined on the basis of the standard curve of bovine serum albumin (BSA) at 25-250 µg mL^{-1} concentrations. All the chemicals used for protein content determination were purchased from Sigma-Aldrich (USA) specification were procured from local chemical vendor.

Protein profile analysis with SDS-PAGE (Sodium dodecyl sulfate-polyacrylamide gel electrophoresis): The method described by Taylor *et al.*[20] was used for SDS-polyacrylamide gel electrophoresis (SDS-PAGE). The procedure consists of sample preparation, making gel (stacking gel and separating gel) and running the gel. The protein profile was identified by comparing the results of the sample electrophoresis with protein. The solution for the separating gel (12%) consisted of 3.35 mL of distilled water, 2.5 mL of tris-hydrogen cloride (Tris-HCl) 1.5 M, 0.1 mL of 10% sodium dodecyl sulfate (SDS), 4 mL acrylamide, 0.05 mL ammonium persulphate (APS) 10% and 0.008 mL tetra methyldiamine (TEMED). The solution for the stacking gel (4.5%) comprised of 2.95 mL of distilled water, 1.25 mL of 1.5 M Tris-HCl, 9.05 mL of 10% SDS, 0.7 mL acrylamide, 0.05 mL of 10% APS and 0.008 mL TEMED. All electrophoresis reagents were purchased from Sigma-Aldrich (USA) specification were procured from local chemical vendor.

The analysis of the protein profiles that appeared in the polyacrylamide gel of SDS-PAGE (Protean II, Bio-Rad, Hercules, USA) was carried out by identifying the molecular weight of the protein bands for each sample by comparing it with the standard for protein markers. The determination of the molecular weight values of the protein bands was based on a molecular weight calibration curve. This was done by determining the migration distance of the sample or the value of the retardation factor (Rf)[21].

Statistical analysis: The data obtained from the research showed trypsin inhibitor activity, protein content and protein profile. To determine the significant difference in trypsin inhibitor activity based on sources of plant tissue, the data was analyzed using multiple factor ANOVA (analysis of variance) with complete randomized design using three factors:

- **Tree age class:** 1-2 years, 3-4 years and 5-6 years
- **Tree condition:** Healthy and gall rust infected sengon twigs
- **Tree tissue source:** Bark and wood from the twigs

If the result of ANOVA showed a significant effect ($p<0.05$), further tests were carried out using Duncan's multiple range test (DMRT) with a confidence significance level of 5%[22].

RESULTS AND DISCUSSION

Trypsin inhibitor activity: Trypsin inhibitor activity of healthy and gall rust infected bark and wood of sengon twigs are presented in Fig. 1. The figure shows that the highest activity of the trypsin inhibitor was found in the gall rust infected bark of sengon twigs aged 1-2 years and the lowest activity was found in the healthy wood of sengon twigs aged 3-4 years. ANOVA revealed that tree tissue source, tree condition and tree age had a significant effect on trypsin inhibitor activity ($p<0.05$). Similarly, the two-factor interaction between the tree tissue sources and tree condition and between tree tissue sources and tree age had a significant effect on the activity of the trypsin inhibitor ($p<0.05$). Meanwhile, the interaction between tree condition and tree age had no significant effect on trypsin inhibitor activity.

Effect of tree condition on trypsin inhibitor activity: Trypsin inhibitor activity in healthy and gall rust infected sengon twigs caused by *U. tepperianum* fungus is presented in Fig. 2. The figure shows that the activity of the trypsin inhibitor in gall rust infected twigs was higher than in the healthy wood. The higher activity of trypsin inhibitors in gall rust infected twigs

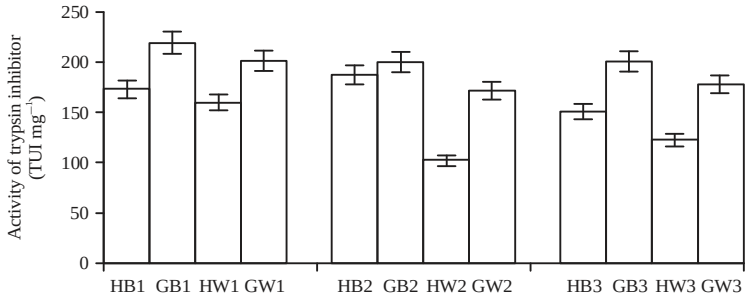

Fig. 1: Trypsin inhibitor activity of health and gall rust-infected bark and wood of sengon tree
HB: Healthy bark, GB: Gall rust-infected bark, HW: Healthy wood, GW: Gall rust-infected wood, 1: 1-2 years, 2: 3-4 years, 3: 5-6 years

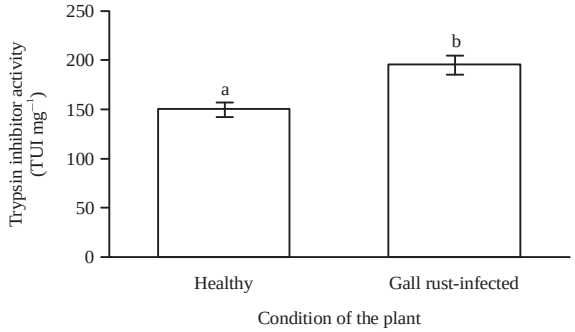

Fig. 2: Trypsin inhibitor activity on sengon twigs in tree conditions

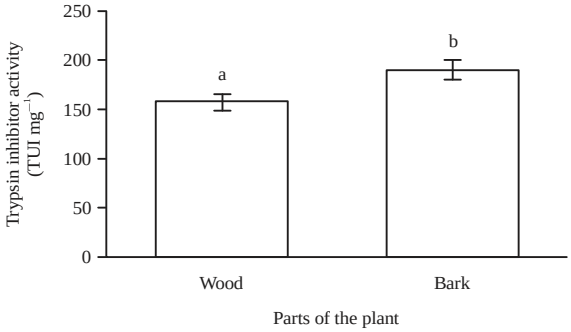

Fig. 3: Trypsin inhibitor activities at tissue parts of sengon tree

is a form of plant self-defense as explained by Prell and Day[23]. Prell and Day[23] suggested that the mechanism of plant resistance is shown in the form of hypersensitive cells through the formation of callose, lignin or structural proteins, phytoalexin compounds and the synthesis of PR proteins such as glucanase, endohydrolase, chitinase and protein inhibitors. In this research, the *U. tepperianum*, produces extracellular protease enzymes (protein breakers). In response to this attack, sengon trees synthesize protease inhibitors, in this case a trypsin inhibitor to suppress the activity of protease enzymes produced by *U. tepperianum*.

Increases in the level of trypsin inhibitors in disease and pest infected plants were also found in several other species, including tomatoes infected by *Phytophthora infestans*[24] and soybean attacked by *Diabrotica virgifera* and *Leptinotarsa decemlineata*[25]. Other researchers also found an increase in the activity of serine inhibitors in potato tubers infected with *P. infestans*[26] and proteinase inhibitors in melon plants infected with *Colletotrichum lagenarium*[27].

Effect of tree tissue sources on trypsin inhibitor activity: The activity of trypsin inhibitors on the wood and bark of sengon twigs is presented in Fig. 3. Based on the Duncan's test, different tissues gave different trypsin inhibitor activities ($p<0.05$). Trypsin inhibitor activity produced by the wood was significantly different from the activity produced by the bark. Figure 3 shows that the trypsin inhibitor activity in the bark tissue was higher than the activity in the wood tissue. This indicates that the bark is the ultimate protection for the tree from pathogen attacks including *U. tepperianum* fungus. The high activity of trypsin inhibitor in the bark is also due to the living cells of tree wood mainly found in the bark so that the activity of the trypsin inhibitor in the bark tends to be higher than in the wood. Higher activity of trypsin inhibitors on the outer part of the plant organ was also found in research conducted by Chaidamsari *et al.*[28], who found that the activity of proteinase inhibitors on the cocoa rind was higher when compared to that in the seeds. Chaidamsari *et al.*[28] suggest that the rind functions as the cocoa's protection system from outside interference, especially from the cocoa fruit borer pest and black pod diseases caused by *Phytophthora palmivora*.

Effect of tree age on trypsin inhibitor activity: The activity of trypsin inhibitors on different trees of different ages is presented in Fig. 4. The figure shows that the highest activity of trypsin inhibitor was found in trees aged 1-2 years, followed by trees aged 3-4 years and 5-6 years. The Duncan's test

suggested the activity of trypsin inhibitors in sengon trees aged 1-2 years was significantly different from those aged 3-4 years and those aged 5-6 years. Meanwhile, the activity of the trypsin inhibitor in sengon trees aged 3-4 years was not significantly different from trees aged 5-6 years.

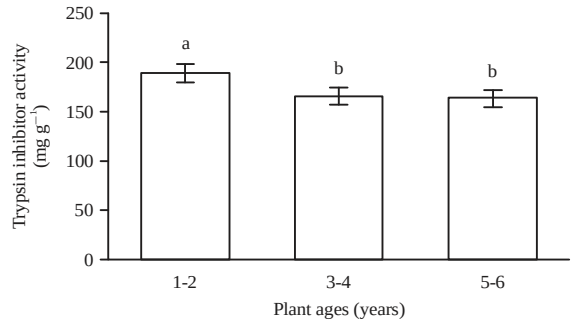

Fig. 4: Trypsin inhibitor activity on sengon tree in different age-classes. The same letter notation on the bar indicates not statistically different at p<0.05 by the Duncan's test

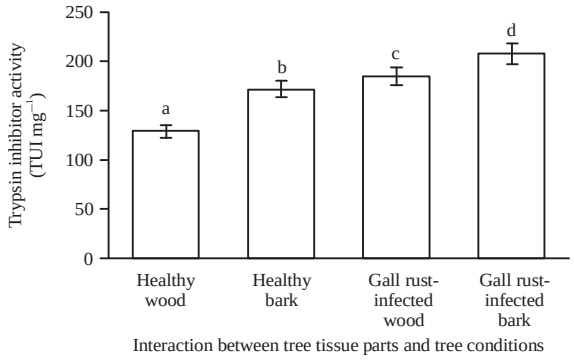

Fig. 5: Trypsin inhibitor activity on interaction between tree tissue parts and tree conditions. The same letter notation on the bar indicates not statistically different at p<0.05 by the Duncan's test

In this study, the higher activity of trypsin inhibitors in sengon trees aged 1-2 years compared with the other tree ages suggests that the severity of gall rust disease is higher in young trees. This is because, in young plants, the fungus can penetrate into the plant tissue rapidly, since the young plants do not yet have a strong defense system against pathogenic infection[29]. Similar results were reported by Hamdayanty and Tri[30], showing that yard long beans infected with BCMV (bean common mosaic potyvirus) at an older age (four weeks after planting) had less severe disease symptoms than those infected with BCMV at a younger age.

Effect of interaction between tree tissue sources and tree conditions on trypsin inhibitor activity: The interaction between the tree tissue source (bark and wood) and the tree condition (healthy or gall rust infected) on the activity of the trypsin inhibitor can be seen in Fig. 5. As the interaction of tree tissue sources and tree conditions have a significant effect on trypsin inhibitor activity, the Duncan test was performed to see differences between the combination of tree tissue source and tree condition and the effects on trypsin inhibition activity. The test result shows that the combined factors, healthy wood, healthy bark, infected wood and infected bark, made a significant difference to the activity of the trypsin inhibitor ($p<0.05$). The highest activity of trypsin inhibitor was found in the infected bark, followed by the infected wood, healthy bark and the lowest activity of trypsin inhibitor was found in the healthy wood.

Effect of interaction between tree tissue source and tree age on trypsin inhibitor activity: Figure 6 shows the trypsin inhibitor activity from the interaction between tree tissue sources (bark and wood of twigs) with tree age (1-2 years, 3-4 years and 5-6 years).

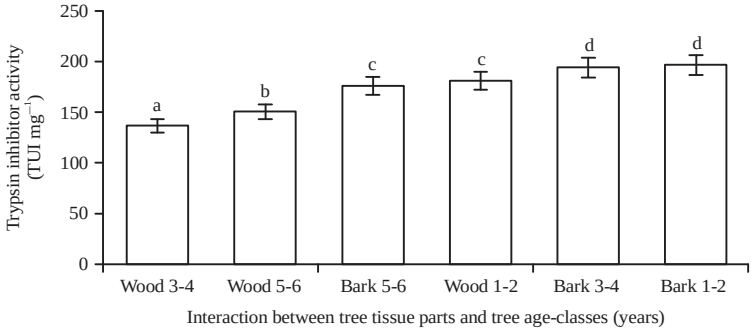

Fig. 6: Trypsin inhibitor activity from the interaction between tree tissue parts and tree age-classes. The same letter notation on the bar indicates not statistically different at p<0.05 by the Duncan's test

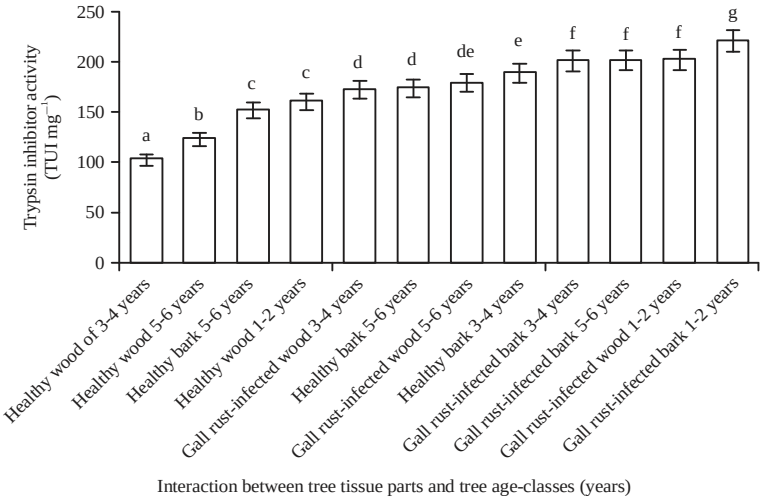

Fig. 7: Trypsin inhibitor activity on tree tissue parts, tree conditions and tree age-class interaction. The same letter notation on the bar indicates not statistically different at $p<0.05$ by the Duncan's test

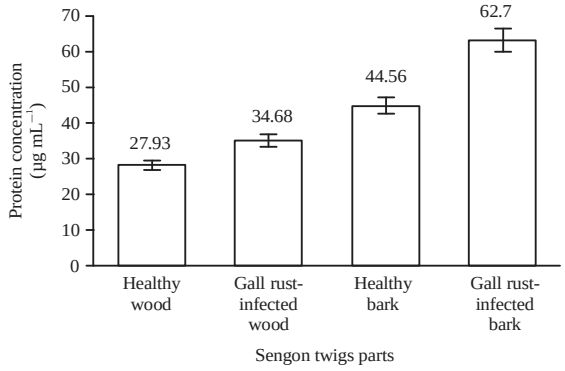

Fig. 8: Protein concentration of healthy and gall rust-infected sengon twigs

It can be seen from this figure that the highest activity of trypsin inhibitor was found in the bark aged 1-2 years and the lowest one was the wood aged 3-4 years. The trypsin inhibitor activity in the wood aged 3-4 years was significantly different from the trypsin inhibitor activity in the wood aged 5-6 years, the bark aged 5-6 years, the wood aged 1-2 years, the bark aged 3-4 years and the bark aged 1-2 years. Similarly, the wood aged 5-6 years also had a distinctly different trypsin inhibitor activity to all the interactions of tree tissue sources with other tree ages. The trypsin inhibitor activity in the bark aged 5-6 years was significantly different from all tree tissue interactions with other tree ages except for the trypsin inhibitor activity of the wood aged 1-2 years. While the activity of the trypsin inhibitor in bark aged 3-4 years was not significantly different to the bark aged 1-2 years, it was significantly different to the activity of trypsin inhibitors at the interaction of tree tissue source and other tree ages ($p<0.05$).

Effect of interaction between tree tissue sources, tree conditions and tree age to trypsin inhibitor activity: Trypsin inhibitor activity produced by different tree tissue sources, tree conditions and tree age can be seen in Fig. 7. The highest activity of trypsin inhibitor was obtained from the interaction between gall rust infected bark aged 1-2 years (219.69 TUI mg^{-1}) and the lowest activity was obtained from the interaction of the healthy wood aged 3-4 years (102.24 TUI mg^{-1}).

The result of the Duncan's test ($p<0.05$) shows that the healthy wood aged 3-4 years, the healthy wood aged 5-6 years and the gall rust infected bark aged 1-2 years had a trypsin inhibitor activity that is significantly different to all other interactions. The activity of the trypsin inhibitor in healthy bark aged 5-6 years was not significantly different to the healthy wood aged 1-2 years but it was significantly different from other trypsin inhibitor activity interaction. The activity of trypsin inhibitors in the gall rust infected wood aged 3-4 years was not significantly different from the healthy bark aged 5-6 years and the gall rust infected wood aged 5-6 years. While the activity of the trypsin inhibitor in the gall rust infected wood aged 5-6 years was not significantly different from the healthy bark aged 3-4 years, it was significantly different to other interactions. The gall rust infected bark aged 3-4 years, the gall rust infected bark aged 5-6 years and the gall rust infected wood aged 1-2 years had trypsin inhibitor activity that was not significantly different to each other but significantly different from other interactions ($p<0.05$).

Protein content: The protein content of extract healthy and gall rust infected twigs of sengon tree was shown in Fig. 8

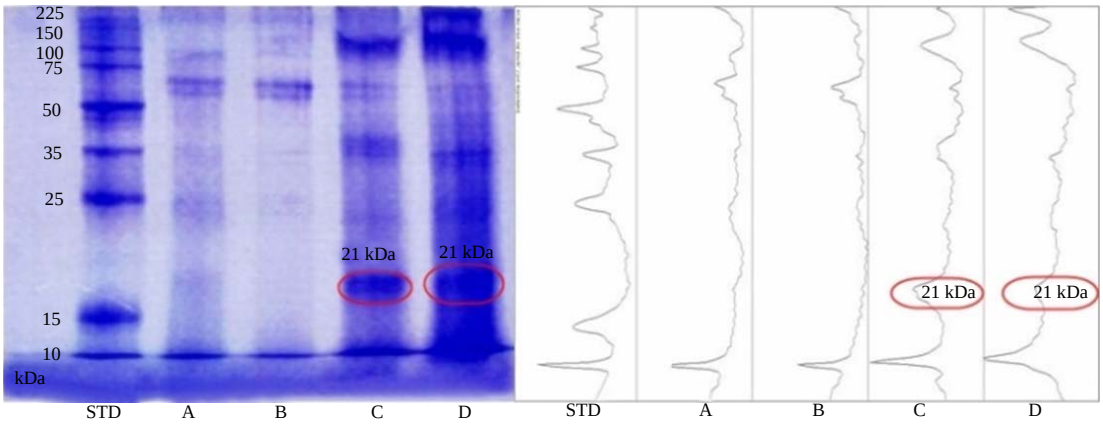

Fig. 9: Result of standard protein electrophoresis and samples. Right: SDS-PAGE results of protein profile electrophoresis (12.5%). Left: ImageJ visualization results of protein profile (STD: Protein Standards/Marker, A: Gall rust-infected wood, B: Healthy wood, C: Gall rust-infected bark, D: Healthy bark)

below. The data on protein concentration of healthy and gall rust infected sengon twigs indicates that sengon trees infected by *U. tepperianum*, that causes gall rust, increases the protein concentration compared to healthy sengon trees. This is because pathogens will naturally increase the synthesis of various proteins in the tree as a defense mechanism to inhibit the development of pathogens. Proteins that are related to the defense mechanism in plants, in general are coined as PR proteins. PR proteins on plants are generally induced by various types of pathogens such as viruses, bacteria and fungi[5].

PR proteins were first identified in a tobacco leaf (*Nicotiana tabacum*) that was infected by the tobacco mosaic virus and subsequently, had been detected in many plants[31]. PR proteins are a group of specific proteins from plants that accumulate after infection. Chitinase, glucanase and peroxidase are some types of PR proteins whose expression increases with the occurrence of infections in the plant tissue. Chitinase and glucanase enzymes produced by plants can hydrolyze chitin and glucan polymers as cell wall components of fungal hyphae, which might inhibit the growth of fungal pathogens[31-34].

The sengon plant's defense mechanism against infection of gall rust disease by *U. tepperianum* fungus is formed by increasing the concentration of defense enzymes, therefore, the protein concentration in infected plants is higher compared to the healthy sengon plant. Several studies have also reported that plant defense mechanisms against pathogens are indicated by the increase of peroxidase activity. This phenomenon happens in several plants like beans infected by *Sclerotium rolfsii*[35], pepper infected with the cucumber mosaic virus,[36] infected *Trichosanthes cucumerina*[37], and infected paddy[38]. Similarly, the research conducted by Diemberg and Peterson[39] stated that soybean infected with gall rust disease caused by *Phakopsora pachyrhizi* increased ammonia lyase enzyme activity.

Protein profile: Protein profile in this study was observed using SDS-PAGE. Figure 9 shows the protein profiles of infected and healthy plant tissues and the concentration of proteins was determined by measuring the color intensity of the bands using Image J software.

Figure 9 shows the different protein profiles between healthy and gall rust infected sengon trees, both the wood and the bark from twigs. The results of molecular weight calculation of protein bands from healthy and gall rust infected sengon on the wood and bark can be seen in Table 1.

Table 1 shows that two protein bands, with a molecular weight of 62.6 and 10 kDa, were detected in healthy wood twigs whereas, six protein bands, with molecular weight of 84, 62.6, 34.9, 24, 21 and 10 kDa, from gall rust infected wood twigs were detected. In addition, seven protein bands, with a molecular weight of 103.2, 62.6, 53.2, 34.9, 24, 21 and 10 kDa, were detected in healthy and gall rust infected bark.

Protein with a molecular weight of 10 kDa was found in wood and bark both healthy and diseased. It means that both healthy and gall rust infected sengon trees express the same protein band profile. Carlberg *et al.*[40] explained that protein with a molecular weight of 9-10 kDa is from the phosphoprotein thylakoid group that acts as the main regulation in all cellular functions, metabolic control and plays an important role in the process of photosynthesis.

Furthermore, a protein with a molecular weight of 21 kDa was found in wood and bark both healthy and diseased. In

Table 1: Molecular weight protein bands profile

Molecular weight (kDa)	Healthy wood	Gall rust-infected wood	Healthy bark	Gall rust-infected bark
103.2	-	-	+++	+++
84.0	-	+	-	-
62.6	++	++	++	++
53.2	-	-	+	++
34.9	-	+	+++	+++
24.0	-	+	+	+
21.0	-	+	+++	+++
10.0	+++	+++	+++	+++

-None, +Very thin, ++Thin, +++Thick

healthy and gall rust infected wood, very thin bands appear, while in the healthy and gall rust infected bark thicker bands appear. According to Albert *et al.*[41] the thickness of the protein bands implies the concentration of protein where a thicker intensity has a higher concentration. In healthy bark, the percentage of the band thickness intensity is 16.61% while in the gall rust infected bark, the percentage of the band thickness intensity is 24.41%. The thickening of the protein bands in the gall rust infected bark is due to the activity of the pathogenic fungus *U. tepperianum* and the response of the sengon tree to the gall rust infection. The 21 kDa molecular weight protein is a trypsin inhibitor (protease inhibitor) which is a sengon tree defense protein against fungus *U. tepperianum* that causes gall rust disease. The results of this study are consistent with the study by Valueva *et al.*[27], who also found that potato infected with *P. infestans* contained defense proteins, that is, trypsin inhibitors with a molecular weight of 21 and 22 kDa.

Protein with a molecular weight of 24 kDa (which is also a trypsin inhibitor found in soybean) is also found, with a very thin intensity expression, in the wood and bark of healthy and gall rust infected sengon trees. Proteins with a molecular weight of 53.2 and 62.6 kDa are found in all parts of the wood and either healthy or gall rust infected bark while proteins with molecular weight of 34.9 and 103.2 kDa are not found in the wood of healthy and diseased trees but with a thick intensity on the bark. Similarly, a protein with a molecular weight of 84 kDa is found with a very thin intensity. According to Supriyadi *et al.*[42] the protein pattern was probably one form of plant reaction to a change in environment to unfavorable circumstances as a safeguard against tissue and protein damage.

CONCLUSION

Sengon trees (*F. moluccana*) infected by *U. tepperianum* fungus, that causes gall rust disease, show increased activity of trypsin inhibitors and protein content that acts as a defense mechanism. The trypsin inhibitor activity n the wood is higher than in the bark. Trees aged 1-2 years have the highest activity of trypsin inhibitor compared to trees aged 3-4 years and trees aged 5-6 years. Proteins with a molecular weight of 21 kDa are expressed in the sengon tree as a trypsin inhibitor.

SIGNIFICANCE STATEMENT

This article discussed the unique trypsin inhibitor that is widely found in plants and acts as a plant defence strategy against pathogens. A gall rust-infected *Falcataria moluccana* by *Uromycladium tepperianum* fungus showed that there was a protein with a molecular weight of 21 kDa which was indicated as a trypsin inhibitor. Infected Sengon tree shows the increasing activity of trypsin inhibitor and protein content that acts as a defence mechanism. I have no similar manuscripts already published from this project.

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