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Physiological resistance responses of rice plant (*Oryza sativa*) provided with silicate fertilizer to sheath blight disease (*Rhizoctonia solani*)

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Abstract. *Azizah R, Rachmawati D. 2023. Physiological resistance responses of rice plant (*Oryza sativa*) provided with silicate fertilizer to sheath blight disease (*Rhizoctonia solani*). Biodiversitas 24: 3785-3795.* Sheath blight disease that is caused by the fungus *Rhizoctonia solani* Kühn generally leads to the reduction of rice yields. The application of silicate fertilizers can increase the resistance to pathogens. This research aimed to determine the effect of silicate fertilizer application on the physiological resistance responses of rice plants to *R. solani*, the primary pathogen responsible for rice sheath blight. This study used three rice cultivars with different resistance levels to sheath blight, including Pandan Wangi (resistant), Cisadane (susceptible), and IR64 (moderately resistant), which were treated with three doses of silicate fertilizer 0 kg ha^{-1}, 200 kg ha^{-1} and 400 kg ha^{-1} with sheath blight inoculation and without inoculation. The results showed that Pandan Wangi provided with 200 kg ha^{-1} silicate fertilizer produced the highest reduction in the percentage of relative lesion height (RLH), and the application of 400 kg ha^{-1} silicate fertilizer had a greater positive effect of reducing the lesion in Cisadane and IR64. In susceptible and moderately resistant cultivars, Cisadane and IR64, higher doses of silicate fertilizer have a better effect on strengthening plant tissue during inoculation treatments. The application of 400 kg ha^{-1} silicate fertilizer without inoculation significantly affected relative water content. In the moderately resistant cultivar IR64, the silicate fertilizer played a role in the physiological response by strengthening the plant tissue, as indicated by the increasing lignin content, sclerenchyma and cuticle thickness.

Keywords: Cultivars, physiological resistance, sheath blight disease, silicate fertilizer

INTRODUCTION

Rice (*Oryza sativa* L.) is a food source plant for almost 50% of the world's population. The rice plant belongs to the genus Oryza and includes approximately 25 species spread in tropical and sub-tropical areas, including Indonesia. Indonesia is an agricultural country with abundant rice yields, but the rice yields significantly decrease caused by pests, disease or weeds (Savary et al. 2000). Sheath blight is one of the main diseases of rice plants. The disease is caused by the soil-borne fungus *Rhizoctonia solani* Kühn (Yang and Li 2012). Rice sheath blight is commonly found in various regions in Indonesia, especially in Central Java. According to Indonesian Center for Rice Research (2009), rice sheath blight is found in Indonesia's highland to lowland rice ecosystems. Sheath blight disease is a serious problem in many rice-growing countries, including Indonesia (Widiantini et al. 2022)

Rice sheath blight can cause symptoms of lesions in the infected area. Spreading lesions makes rice plant stalks soft and brittle, causing the stems easily fall and obstruct the flow of nutrients. As a result, rice grain is not optimally filled (Nuryanto 2018; Raj et al. 2019). The filling of rice grains that is not optimal causes a drastic reduction in rice plant productivity due to a large number of empty rice grains. Rice sheath blight will cause a significant reduction in yields. The rice sheath blight pathogens can cause damage to rice plants and reduce yield by 50-80% (Rosmaladewi et al. 2020). Therefore, some efforts are needed to overcome this problem, one of which is by increasing the resistance of rice plants.

Plants' resistance can be seen based on plants' response in defending against biotic and abiotic stress. The form of plant resistance response when facing biotic stress can be in the form of a physical response that can be seen structurally (physical defense) and biochemically (chemical defense). The physiological response is defined structurally by the deformation a defense barrier in the form of a cuticle or cellulose in the epidermal tissues to strengthen the cell walls of the plants (Seal et al. 2018). While the biochemical response (chemical defense) of plants provides the plants with a defense mechanism, one of which is to produce secondary metabolites in the form of lignin (Leroy et al. 2019; Seal et al. 2018), which accumulates in the cell walls of plant tissues. Plant lignin contributes to the plant cell wall by providing strength and resistance, allowing the cell to become more rigid and serve as a barrier to protect plants from pathogenic fungi.

The response of plant resistance to pathogens, especially in rice plants, can be improved using silicate fertilizers. Silicon (Si) is one of the beneficial elements needed by some plants. Silicon is also recognized as an essential nutrient needed by rice plants. The silicon accumulation in rice plants can increase resistance and increase the growth and productivity of rice plants (Seal et al. 2018). Silicon plays an important role in the formation of plant tissue, especially in increasing the strength of the tissue by forming a structural barrier in the form of silica-cuticle and silica-cellulose. Si plays an active role in increasing plant resistance to various infectious disease-causing pathogens (Ma and Yamaji 2006; Seal et al. 2018). In addition, silicon plays a role in inducing lignin accumulation and activating the defense mechanism of rice plants from the attacking pathogen (such as *R. solani*), which causes rice sheath blight.

Based on this background, a study was conducted to determine the physiological responses as a defense mechanism in rice plants by applying silicate fertilizers against infection of rice leaf sheath blight (*R. solani*.). The beneficial silicon element's important role in increasing plant resistance against leaf sheath blight can be defined by observing physiological, biochemical, and anatomical parameters.

MATERIALS AND METHODS

Experimental design

The study was a factorial experiment (three factors) conducted using a completely randomized design. The treatments consisted of the application of silicate fertilizer dosages and inoculation of the fungus *R. solani* on three rice cultivars of Pandan Wangi (sheath blight-resistant), Cisadane (sheath blight-susceptible) and IR64 (sheath blight-moderately resistant). Three doses of silicate fertilizer (0,200 and 400 kg ha^{-1} of $CaSiO_3$) were applied in this study. The combination of rice cultivars and doses of silicate fertilizer were treated with and without inoculation of the fungus *R. solani*.

Procedure

Application of silicate fertilizer and inoculation of the fungus Rhizoctonia solani

Rice seeds from three cultivars were soaked for 48 hours and germinated in pot trays. The seven days old seedlings were transplanted into the experimental pot containing 1 kg of growing media (soil and compost with a ratio of 3:1, respectively). The silicate fertilizer application with doses of 0 kg ha^{-1}, 200 kg ha^{-1} (0.08 g pot^{-1}) and 400 kg ha^{-1} (0.16 g pot^{-1}) were applied two days before transplanting. Inoculation treatment was conducted when the rice plants' age was 40 days after planting (DAP) (Azizah 2022). Isolates of *R. solani* were obtained from the Department of Pests and Plant Diseases, Faculty of Agriculture, Universitas Gadjah Mada. Inoculation of the *R. solani* fungi was done using the aluminum foil and micro-chamber method based on Jia et al. (2013), which was modified at the stage of making *R. solani* inoculum (Figure 1).

Parameters measured include physiological parameters consisting of relative lesion height (RLH) based on the Standard Evaluation System for Rice (IRRI 2002), relative water content (Barrs and Weatherley 1962), and chlorophyll content (Harborne 1987), anatomical parameters which include the diameter of the leaf sheath, the thickness of the cuticle, and the thickness of sclerenchyma tissue, as well as biochemical parameters including the lignin content was measured using Klason Method based on TAPPI T 22 with modification on preparation of cell wall protein fractions (Moreira-Vilar et al. 2014).

Relative Lesion Height (RLH)

The rice plants were inoculated 40 days after planting (DAP). The sheath blight disease symptoms appeared seven days after inoculation (DAI); the lesion was measured and counted to determine the percentage of blight disease symptoms in the form of relative lesion height (RLH) percentage. RLH was measured based on the Standard Evaluation System for Rice (IRRI 2002). The blight symptoms percentage of RLH was used to count the damage level of pathogen infection by measuring the length of the infection area (lesion) and the plants' height using the following formula:

$$RLH (\%) = (\text{Lesion height: plant height}) \times 100\%$$

The RLH was scored, and the rice plant was classified as follows: (i) 0: Highly resistant: no infection, (ii) 1: Resistant: lesion height is less than 20% of the plant height. (iii) 3: Moderately resistant: lesion height is between 20-30% of the plant height. (iv) 5: Moderately susceptible: lesion height is between 31-45% of the plant height. (v) 7: Susceptible: lesion height is between 46-65% of the plant height. (vi) 9: Highly susceptible: lesion height is more than 65% of the plant height.

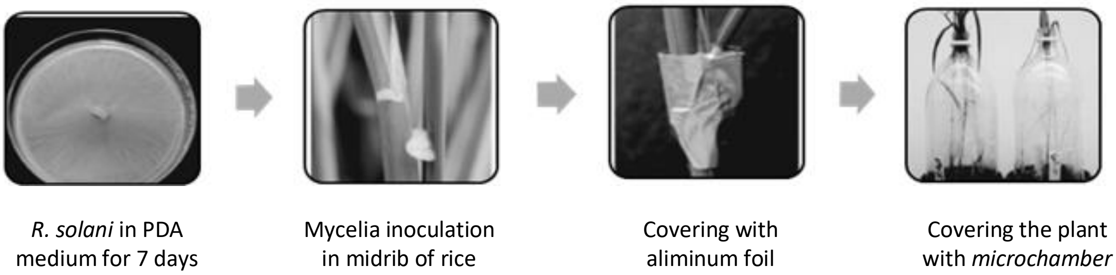

Figure 1. Aluminum foil method with micro-chamber (Jia et al. 2013; Park et al. 2008)

Relative Water Content (RWC)

The percentage of relative water content in leaves was measured based on the method of Barrs and Weatherley (1962). The youngest fresh leaves were taken for 1 cm on one-third of the leaf's length from the tip. The sample was weighed using an analytical scale to determine the fresh weight (FW), immersed in distilled water at 25°C for 4 hours and reweighed to determine the turgid weight (TW). Then the leaf sample was oven-dried (80°C for 24 hours) for the dry weight (DW). The results of the calculation of FW, TW, and DW were used to determine the relative water content in the leaves using the following formula:

$$RWC\ (\%) = \left[\frac{FW-DW}{TW-DW}\right] \times 100\%$$

Where:
RWC : relative water content (%)
FW : Fresh weight/ Wet weight (g)
DW : Dry weight (g)
TW : Turgid fresh weight (g)

Chlorophyll content

The chlorophyll content was determined by the spectrophotometry method (Harborne 1987). Fresh infected rice leaf tissue was taken and then weighed using an analytical scale until it reached a weight of 0.1 grams. The sample was crushed using a porcelain mortar and dissolved in 10 mL of 80% acetone. The solution was vortexed for 10 seconds. The extract was put in a 5 mL centrifuge tube as much as 2 mL, centrifuged for 10 minutes at a speed of 4000 rpm at 4°C, and then filtered using filter paper. The filtrate is accommodated in a test tube. The repetition was done three times. The chlorophyll content was measured by putting 1 mL of the sample solution into the cuvette. The spectrophotometer was calibrated using 80% acetone before use. The absorbance of each extract was measured with a wavelength of 663 nm and 646 nm. The absorbance on the spectrophotometer was used to calculate the chlorophyll content with the following calculations:

$$\text{Total of chlorophyll content} = (17.3\ A_{646}) + (7{,}18\ A_{663})\ \text{mg g}^{-1}$$

Lignin content

Lignin content was measured using the Klason method based on TAPPI T 22 with modification on the preparation of cell wall protein fractions (Moreira-Vilar et al. 2014). The material preparation stage was carried out by cleaning the rice leaves and then cutting the samples with a 3-5 cm length. Samples were dried using an oven at 60˚C until a constant weight was reached. The dried samples (0.3 g) were crushed using mortar and pestle to be a fine powder homogenized in 50 mM potassium phosphate buffer (7 mL, pH 7) and then poured into a centrifuge tube. The pellets were centrifuged (1,400 x g, 5 minutes) and washed with successive stirring, and centrifuged with the following reagents: 2x with phosphate buffer (pH 7, 7 mL), 3x with 1% (v/v) Triton X-100 in buffer pH 7 (7 mL), 2x with 1 M NaCl in buffer pH 7 (7 mL), 2x with distilled water (7 mL) and 2x with acetone (5 mL). The pellets were dried in an oven (60°C, 24 hours) and cooled in a desiccator. The dry material was defined as the protein fraction of the cell wall (Moreira-Vilar et al. 2014).

The 0.1 g of the dry fraction sample was put into a 250 mL beaker and added with 10 mL of 72% H_2SO_4 at a temperature of 47°C while stirred using a stirring rod for 10 minutes. The sample was then diluted to a concentration of 3% acid. Hydrolysis was continued by heating the solution in Erlenmeyer until it boiled. The solution was allowed to cool and let stand until the lignin precipitate settled completely. The cooled sample is then filtered with filter paper with known weight to separate the soluble (solution) and insoluble (precipitate) fractions. The lignin was washed and precipitated until it was free from acid with hot water. Dry lignin in filter paper was precipitated in an oven at 105°C, cool and weighed until constant (Badan Standardisasi Nasional Indonesia 2008; Nawawi et al. 2019). Lignin content was determined using the method of Klason following the formula:

$$x = \frac{A}{B} \times 100\%$$

Where:
x : lignin content value is stated in percent %
A: weight of the lignin precipitate is stated in grams (g)
B: weight of the oven-dry sample is expressed in grams (g)

Anatomical characters

The anatomical cross-section of the leaf sheath was prepared using a semi-permanent method with safranin staining. The anatomical parameters, including the leaf sheath's midrib diameter, the cuticle's thickness, and the sclerenchyma tissue's length and thickness, were measured using a binocular microscope by Optilab and Image Raster Software.

Data analysis

The data of this study consisted of physiological parameters and anatomical parameters. Qualitative data was presented in descriptive form, while quantitative data were analyzed using ANOVA at a 95% confidence level and were further analyzed using the Duncan test at a 95% confidence level if there was a significant difference.

RESULTS AND DISCUSSION

Relative Lesion Height (RLH)

The results showed a significant ($p < 0.05$) interaction between silicate fertilizer and the cultivars on RLH. In general, silicate fertilizer application reduces RLH value in the three tested cultivars (Table 1). Based on the RLH in the inoculated cultivars without silicate fertilizer (Table 1) showed that Pandan Wangi (sheath blight-resistant) had the lowest RLH compared to the other two cultivars (IR64 and Cisadane). The application of silicate fertilizer reduced the percentage of RLH in rice plants infected by *R. solani*. The RLH value in the Pandan Wangi cultivar treated with 200 kg ha^{-1} was lower than 400 kg ha^{-1} of silicate fertilizer. The presence of 200 kg ha^{-1} silicate fertilizer reduced up to 75.51% of the RLH, whereas the silicate fertilizer dose of

400 kg ha^{-1} reduced 55.44% of the RLH in the Pandan Wangi cultivar. Meanwhile, silicate fertilizer application at a dose of 400 kg ha^{-1} had a greater favorable impact than 200 kg ha^{-1} on reducing the RLH value of Cisadane and IR64 cultivars. This treatment can reduce 63.10% and 69.11% of the RLH for IR64 and Cisadane, respectively (Table 1).

Chlorophyll content

The results showed a significant ($p < 0.05$) interaction between inoculation, cultivars and silicate fertilizer on the chlorophyll content. In all cultivars, inoculation of *R. solani* changed the chlorophyll content. The result showed that chlorophyll content in inoculated plants was lower than without inoculation in Pandan Wangi, IR64, and Cisadane (Figure 2). In the sheath blight inoculation treatment, the chlorophyll content increased in line with the increasing dose of silicate fertilizer in all cultivars. However, in the condition without inoculation, plants responded differently to silicate fertilizer application depending on genotypes. In the Pandan Wangi and Cisadane cultivars, the chlorophyll content of plants treated with a dose of 200 kg ha^{-1} was higher than 400 kg ha^{-1} silicate fertilizer. While in the IR64 cultivar, the chlorophyll content of plants treated with silicate fertilizers at a dose of 200 kg ha^{-1} was lower than those treated at a dose of 400 kg ha^{-1}.

Relative Water Content (RWC)

Based on variance analysis ($p < 0.05$) the interaction of rice cultivars, silicate fertilizer and sheath blight inoculation were significantly on relative water content (RWC). All plants inoculated with *R. solani* had less relative water content than the non-inoculated ones (Figure 3). Application of silicate fertilizer at a dose of 200 kg ha^{-1} significantly increased the relative water content in Pandan Wangi and Cisadane cultivars, but the trend tended to decrease with the increasing doses of silicate fertilizer to 400 kg ha^{-1}. The silicate fertilizer application enhanced the relative water content in cultivar IR64, but there was no difference between the inoculation and non-inoculation treatments. Without silicate fertilizer, plants inoculated with *R. solani* had decreased relative water content (less than 70%) and showed early plant wilt.

Lignin content

Silicate fertilizer application and sheath blight inoculation have an impact on lignin content. Based on variance analysis, there was a significant ($p < 0.05$) interaction effect between the combination of silicate fertilizer, inoculation treatment and cultivars on lignin content. In all cultivars, pathogen inoculation and application of silicate fertilizer increased lignin content (Figure 4). In the Pandan Wangi cultivar without inoculation, the application of 200 kg ha^{-1} silicate fertilizer increased the lignin content, but at a dose of 400 kg ha^{-1}, the lignin content decreased. It was demonstrated that silicate fertilizer application raised lignin content in the IR64 cultivar in the inoculation and without inoculation treatments, in which the greater impacts were shown at a dose of 200 kg ha^{-1}. The application of 400 kg ha^{-1} of silicate fertilizer significantly increased the lignin levels in the inoculated plants of the Cisadane cultivar, even higher than that of non-inoculated plants.

Table 1. The RLH and the RLH reduction percentage in the presence of silicate fertilizer on three rice cultivars inoculated with *Rhizoctonia solani*

Cultivars	RLH (%)				RLH reduction (%)			
	Silicate fertilizer (kg ha^{-1})			Average	Silicate fertilizer (kg ha^{-1})			Average
	0	200	400		0	200	400	
Pandan Wangi	13.60±1.0^{d}	3.33±0.9^{a}	6.06±2.0^{b}	7.67^{p}		75.51	55.44	65.48
IR64	15.53±0.5^{d}	7.07±1.5^{bc}	5.73±0.3^{b}	9.44^{q}		54.48	63.10	58.79
Cisadane	19.10±2.0^{e}	8.46±0.7^{c}	5.90±0.7^{b}	11.16^{r}		55.71	69.11	62.41
Average	16.08^{y}	6.28^{x}	5.90^{x}			61.9^{x}	62.55^{x}	

Note: The numbers followed by the same letter in the RLH parameter were not significant difference based on DMRT α = 0.05 n = 3, The RLH reduction percentage indicates the delta value between control (without silicate fertilizer) and silicate fertilizer treatment (200 and 400 kg ha^{-1})

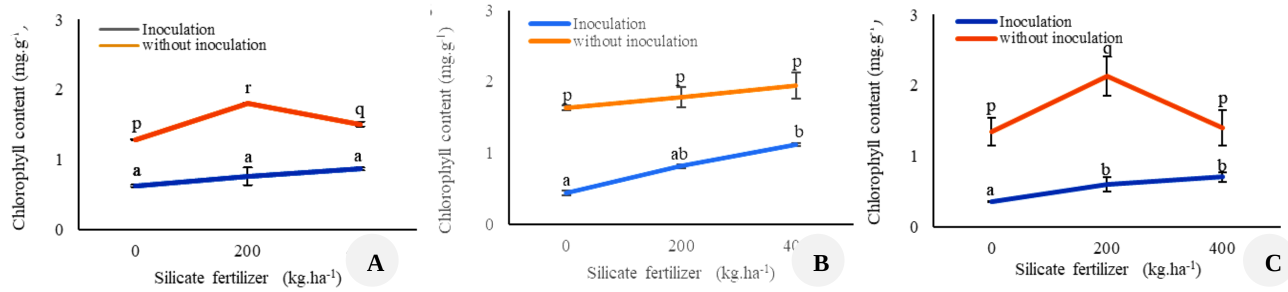

Figure 2. Chlorophyll content on A. Pandan Wangi, B. IR64 and C. Cisadane cultivars treated with silicate fertilizer and inoculation of the sheath blight pathogen *Rhizoctonia solani*. The mean value followed by the same letter has no significant difference based on DMRT α = 0.05

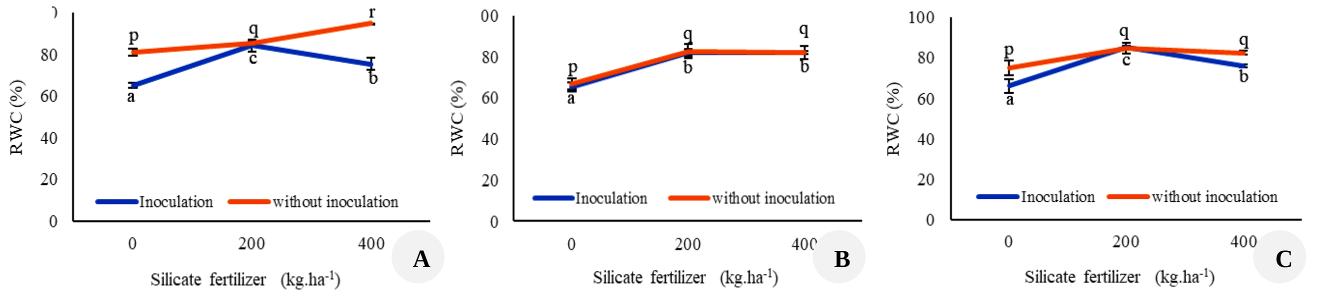

Figure 3. Relative water content (RWC) on A. Pandan Wangi, B. IR64 and C. Cisadane cultivars treated with silicate fertilizer and inoculation of the sheath blight pathogen *Rhizoctonia solani*. The mean value followed by the same letter has no significant difference based on DMRT α = 0.05

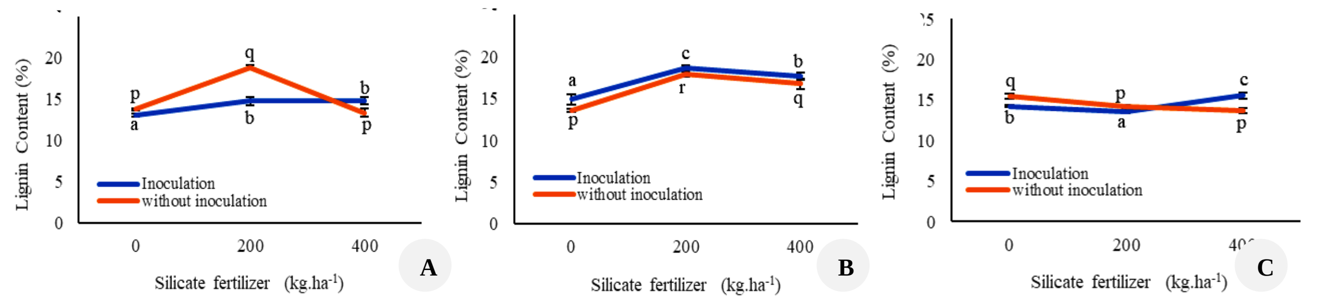

Figure 4. Lignin content on A. Pandan Wangi, B. IR64 and C. Cisadane cultivars treated with silicate fertilizer and inoculation of the sheath blight pathogen *Rhizoctonia solani*. The mean value followed by the same letter has no significant difference based on DMRT α = 0.05

Anatomical characters

The interaction of rice cultivars, silicate fertilizer and sheath blight inoculation significantly ($p < 0.05$) influenced anatomical characters, including sheath diameter, cuticle and sclerenchyma thickness. Regarding fertilizer dose, the plants with silicate fertilizer had a thicker sheath diameter than plants without silicate fertilizer application. In all cultivars, the average sheath diameter with fertilizer treatment at a dose of 400 kg ha^{-1} was smaller than that at 200 kg ha^{-1} (Figure 5). This indicated that the silicate fertilizer at a dose of 200 kg ha^{-1} positively affects the sheath diameter. In the Pandan Wangi cultivar, the sheath diameter of plants without inoculation was almost identical to inoculated plants. However, the Cisadane and IR64 cultivars had larger sheath diameters in the inoculated plants with silicate fertilizer 200 kg ha^{-1} than the non-inoculated plants.

The silicate fertilizer affected the cuticle thickness. Generally, plants treated with silicate fertilizer had a thicker cuticle thickness (Figures 5 and 6). The cuticle thickness in plants treated with silicate fertilizer of 200 kg ha^{-1} was in the range of 4.17-4.96 µm, and in plants treated with silicate fertilizer of 400 kg ha^{-1} was in the range of 3.42-4.73 µm. Nevertheless, the trend of cuticle thickness varied among cultivars with or without sheath blight inoculation. Pandan Wangi and Cisadane cultivars treated with 200 kg ha^{-1} silicate fertilizer had thicker cuticles than 400 kg ha^{-1}. In contrast to Cisadane cultivars, where the cuticle thickness was almost the same in control and inoculated plants, the inoculated plants of Pandan Wangi exhibited thicker cuticles than those without inoculation. Cuticle thickness increased with the increasing dose of silicate fertilizer on the IR64 cultivar without inoculation, whereas in the inoculation treatment, cuticle thickness increased at a dose of 200 kg ha^{-1} but thinner at a dose of 400 kg ha^{-1}.

The thickness and length of sclerenchyma are different in each cultivar (Figure 7). In Panda Wangi and IR64 cultivars with inoculation, silicate fertilizer application significantly reduced the sclerenchyma length. Whereas in the Cisadane cultivar, silicate fertilizer application increased the length of sclerenchyma. In all cultivars without inoculation, silicate fertilizer application showed a decrease in the length of sclerenchyma. Applying 400 kg ha^{-1}, silicate fertilizer increased the sclerenchyma thickness of the inoculated plants in all cultivars. Without silicate fertilizer, the sclerenchyma of inoculated plants was thinner than the healthy plants without sheath blight inoculation. The moderate and susceptible cultivars, IR64 and Cisadane, exhibited the same trend where silicate fertilizer application either in a dose of 200 kg ha^{-1} or 400 kg ha^{-1} resulted in the thicker sclerenchyma tissues in the inoculation treatment.

Discussion

The infection of the sheath blight pathogen caused by *R. solani* showed the symptoms of lesions, i.e., necrotic or chlorotic, localized in the infected area (Figure 8). The pathogen *R. solani* causes lesions, which have morphological traits of being greenish-grey in the infected area. The presence of lesions on the sheath and leaf blade causes rotting and drying of the entire leaf. The leaves' color gradually turns yellow as they dry, eventually reaching an early senescence stage. The yellow leaves occur due to chlorophyll fluorescence or chlorophyll degradation in areas infected with pathogens. Chlorophyll content in plants with inoculation is lower than in plants without inoculation because of chlorophyll degradation. Chlorophyll degradation is used to indicate photosynthesis regulation and plant response to stress (Lin et al. 2018). The lesion will rapidly spread and infect the host if the environmental factors are favorable (Senapati et al. 2022). Necrotic damage, a relatively quick response to pathogen invasion known as programmed cell death (PCD), is one of the plant's defense systems (Minina et al. 2013).

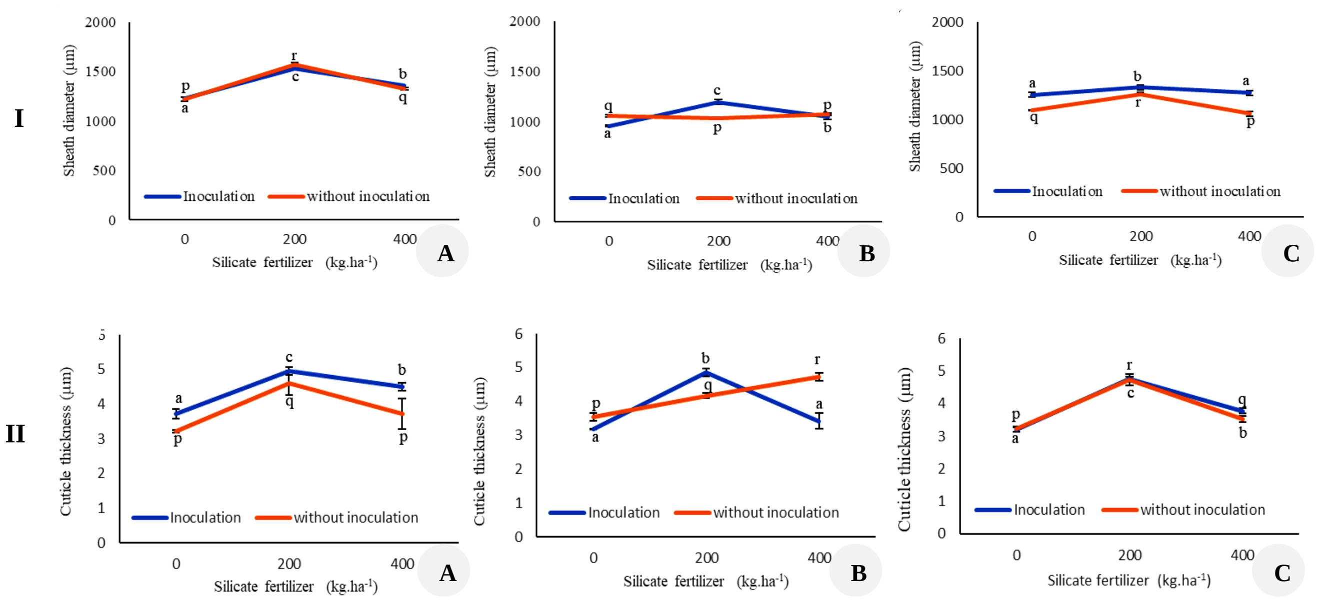

Figure 5. I. Sheath diameter and II. cuticle thickness on A. Pandan Wangi, B. IR64 and C. Cisadane cultivars treated with silicate fertilizer and inoculation of the sheath blight pathogen *Rhizoctonia solani*. The mean value followed by the same letter has no significant difference based on DMRT α = 0.05

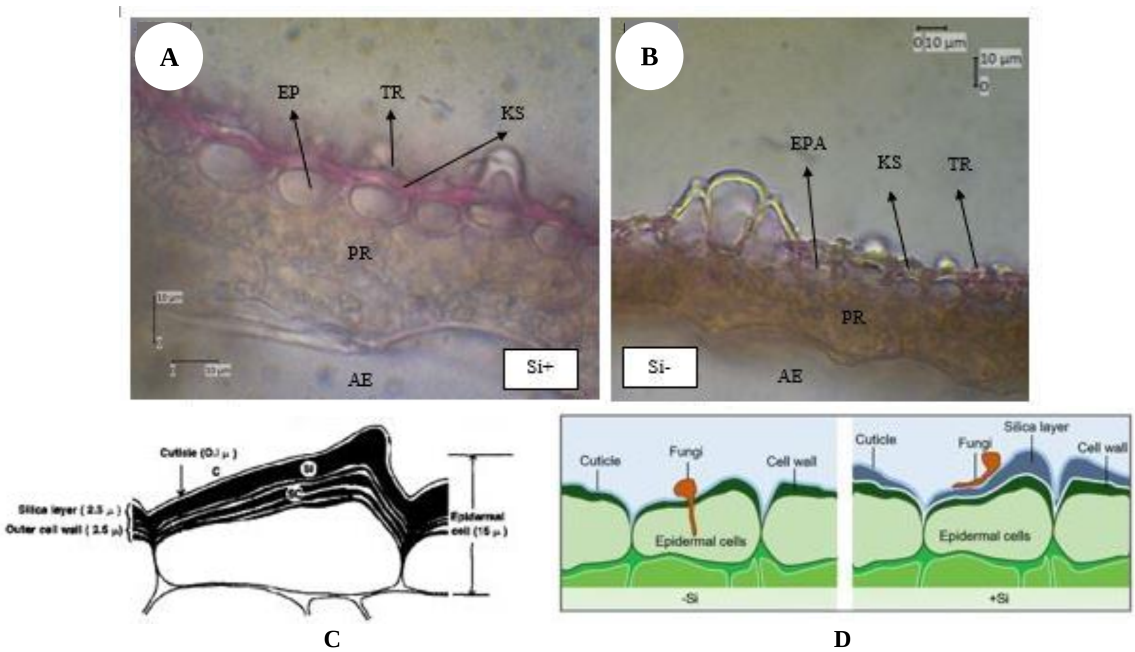

Figure 6. A. Epidermis and cuticle-silica cell layers in plants with silicate fertilizer, B. Epidermal layers of plants without silicate fertilization, C. Cuticle-silica double layers on leaf blades (Rao and Susmitha 2017), D. Illustration Schematic Si- and Si+ Epidermis Layer (Wang et al. 2017). Note: EP: Epidermis, TR: Trichome, KS: Cuticle-Silica, PR: Parenchyma, AE: Aerenchym

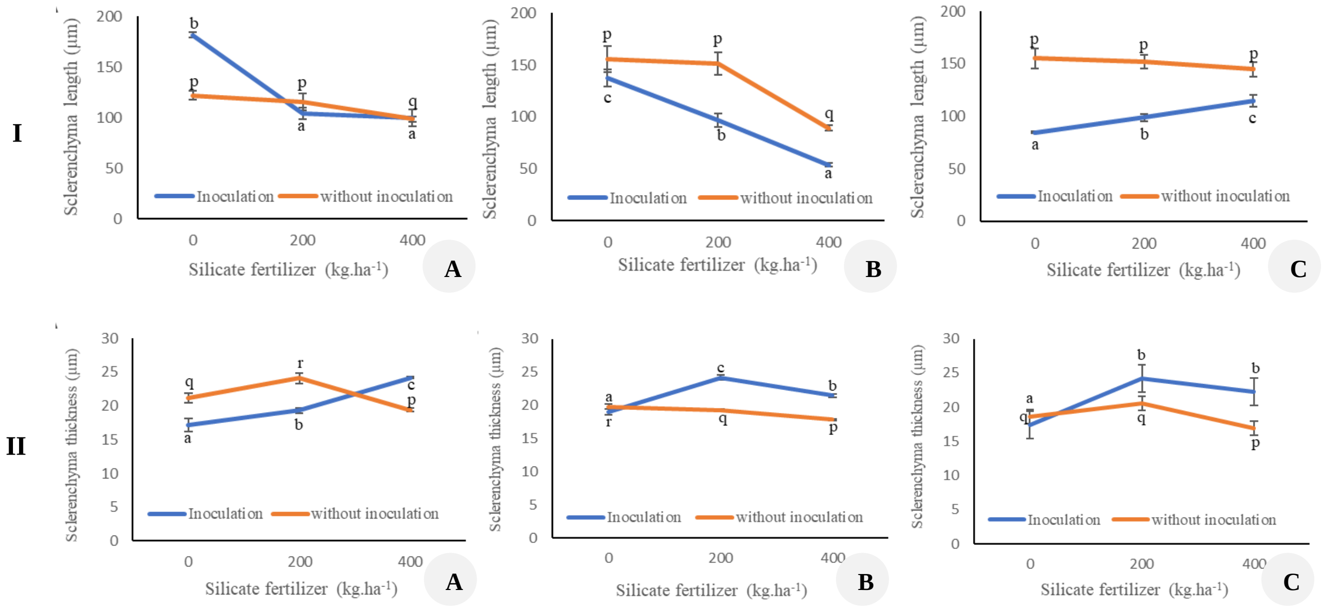

Figure 7. I. Sclerenchyma length and II. Sclerenchyma thickness on A. Pandan Wangi, B. IR64 and C. Cisadane cultivars treated with silicate fertilizer and inoculation of the sheath blight pathogen *Rhizoctonia solani*. The mean value followed by the same letter has no significant difference based on DMRT α = 0.05

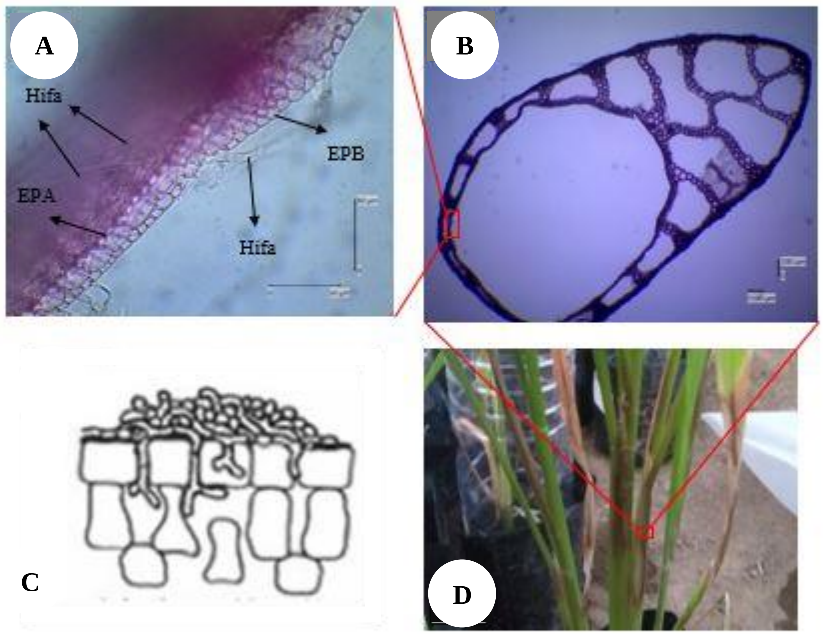

Figure 8. A. The damaged epidermal cell layer by pathogens, B. Cross section of rice sheath, C. *R. solani* fungal penetration schematic (Taheri and Tarighi 2011), D. Lesion on infected rice sheath. Note: EPA: Upper Epidermis, EPB: Lower Epidermis

The appeared symptoms of the lesions based on RLH percentage indicate the severity of the pathogen infection. The more severe the fungal infection of rice sheath blight is, the higher percentage of RLH. Severe infection indicates that the plant is more susceptible to pathogen infection. On the other hand, the lower the RLH percentage, the fewer symptoms of lesions appear, and the plant is more resistant to sheath blight pathogens (IRRI 2022). All the cultivars showed that the RLH percentage was less than 20%. A low RLH value in this study might be due to several factors, including the duration of the pathogen infection. Santosh et al. (2019) reported that the length of the lesion was assessed after 7 and 15 days of inoculation, but the relative length of the lesion after 15 days of inoculation is higher than seven days of inoculation. If the plant is infected with a pathogen for a long duration, the length of the lesion is also higher. In this research, RLH was observed seven days after inoculation because, at the seven days after inoculation, each cultivar gave the resistance response basis for the presence of the pathogen (Irawati and Hartati 2011). Santosh et al. (2019) also reported that the method and inoculation techniques affect RLH scores. The relative number of lesions compared to the control was found to be the maximum lesion in the mycelial suspension spray technique, and the minimum relative number of lesions was recorded in mycelial ball inoculation (Santosh et al. 2019). Mycelial ball produced the longest lesions on rice sheaths after seven days of inoculation than mycelial suspension because the mycelium in mycelial suspension has been broken apart, preventing the allocation of nutrients through the mycelial network to the point of infection (Park 2008). Genetic and environmental factors influence plant resistance to pathogens. Environmental factors such as sunlight, humidity, temperature, soil fertility and silicon level in soil may contribute to the variation in the disease resistance phenotype and make evaluation difficult (Park 2008).

The type of cultivar affects a plant's ability to withstand biotic stress. According to Suprihatno et al. (2010), Cisadane is a susceptible cultivar to sheath blight pathogen. The IR64 cultivar has a high potential to be resistant to brown leafhoppers and is also moderately resistant to leaf blight, while the Pandan Wangi is a superior cultivar that is resistant to sheath blight pathogens (Sitaresmi et al. 2013). This study revealed that Pandan Wangi had the lowest RLH value of 13.60%, followed by IR64 at 15.53%, and Cisadane exhibited the highest RLH of 19.10%. This result is consistent with the finding of Sari (2019), who reported that sheath blight relatively affects the Pandan Wangi cultivar at a disease severity intensity below 30%. Breeding rice cultivars that are tolerant to blight is a feasible control strategy, but due to high pathogen variability and rapid pathogen evolution, the resistance of cultivars with 1 or 2 main resistance genes is unstable in the field (Song et al. 2016). The research showed that silicate fertilizer reduced the RLH by more than 50% in all cultivars. The highest RLH reduction reaching 75.51%, was exhibited by Pandan Wangi, treated with 200 kg ha^{-1} silicate fertilizer. The RLHs of IR64 and Cisadane cultivars were reduced to 54.48% and 55.71%, respectively, in the presence of 200 kg ha^{-1} silicate fertilizer. The percentage of the RLH reduction for IR64 and Cisadane at a dose of 400 kg $.ha^{-1}$ was 63.10% and 69.11%, respectively. Therefore, a combination of external factors is needed to increase plant resistance through silicate fertilizer applications.

A disease will occur if the host interacts with a virulent pathogen and a favorable environment. Nutrients impact a plant's ability to withstand pathogens. Nutrient status, such as soil mineral content, can be manipulated by fertilization. In highly resistant plants, nutrients have a relatively small effect because they possess resistance genes to respond to the pathogen attack. Plants compatible with pathogens will cause a higher disease intensity, but disease-tolerant plants can still survive (Akhsan et al. 2019). The ability of a pathogen to infect the host occurs after the plant has implemented a defense mechanism. Nutrients will affect moderate susceptibility and moderate resistance (Pokhrel 2021). This aligns with the present study findings, which show that Pandan Wangi, a resistant cultivar, has physiological parameters better than those of Cisadane, a susceptible cultivar, and IR64, a moderately resistant cultivar (Table 1).

The application of silicate fertilizer affects increasing plant resistance to pathogens. The results showed that in inoculation treatment, a higher dose of silicate fertilizer has a more positive effect on reducing the lesion by strengthening the plant tissue, such as lignin content (Figure 4), cuticle thickness (Figure 5) and sclerenchyma thickness (Figure 7) in moderate and susceptible cultivar like IR64 and Cisadane. Silicate fertilizer in the form of calcium silicate ($CaSiO_3$) as fertilizer applied to the soil positively reduces the severity of plant diseases caused by pathogens. In general, the effect of silicon on plant resistance is due to silicon deposition in the cell wall area, which acts as a physical defense that it is difficult for pathogens to penetrate and infect plants (Sakr 2016a). According to research conducted by Chang et al. (2002), who treated two doses of silicate on four cultivars with different resistance to blast disease, silicon application significantly reduced lesion length by 5-22%, and research conducted by Xue et al. (2010) regarding the severity of rice plants given the application of silicon was also reduced to 11.83-52.12%. This study exhibited that silicate fertilizer significantly reduced more than 50% of RLH in all cultivars (Table 1). According to an anatomical study, cuticle thickness increased with silicate fertilizer at a dose of 200 kg ha^{-1} (Figure 5). Silicon deposition triggers thickening in the cuticle area as a physical defense mechanism. Thickening of the cuticle occurs due to the cuticle-Si double-layer formation. Silicon accumulates and tends to be deposited on the leaf surface, leaf blade, and leaf sheath as a layer approximately up to 2.5 µm of thickness below the thin cuticle layer to form a cuticle-silicon double layer which helps plants to maintain strengthand cell rigidity, minimize transpiration and protect plants from diseases and insects (Rao and Susmitha 2017; Wang et al. 2017; Sathe et al. 2021). The inoculated plants become thicker, presumably due to a defense mechanism against pathogens by forming a physical barrier. The physical barrier is a thickening of the sclerenchyma tissue through the lignification process of sclerenchyma cells near the epidermis induced by the *OsMYB30* gene (Li et al. 2020) and forming a cuticle-Si double layer due to induction by silicate fertilizers (Rao and Susmitha 2017; Wang et al. 2017; Sathe et al. 2021). Both physical barriers have the same goal of preventing the penetration of pathogenic fungi.

Rice plants absorb Si in a fertilizer dose range of 230-470 kg ha^{-1} or about twice higher than nitrogen uptake (Rao et al. 2017; Subiksa 2018). The element of Si also plays a role in increasing the availability of nutrients, including nitrogen elements (Rao et al. 2017). Nitrogen is one of the essential nutrients for growth and development and the main component of chlorophyll and protein, which is closely related to leaf color in agricultural plants such as rice plants (Wang et al. 2014). According to Yoshida (1981), rice plants' ability to erect leaves increases the photosynthetic efficiency of plants when given silicate fertilizers rather than nitrogen fertilizers in tropical lowland areas because the Si element causes rice plants to have more upright leaves, allowing photosynthesis to run smoothly. Si can increase the role of leaves as photosynthetic organs by up to 10% (Rao et al. 2017). The application of silicate fertilizer to the three cultivars showed a tendency to increase the relative water content, which was higher in the treatment without inoculation. This finding is consistent with the result by Wang et al. (2017), plants with silicate fertilizers had higher relative water content due to the Si element increased the strength of the tissue and cell walls and increased the elasticity of the cell walls so the plants became more upright and did not fall easily.

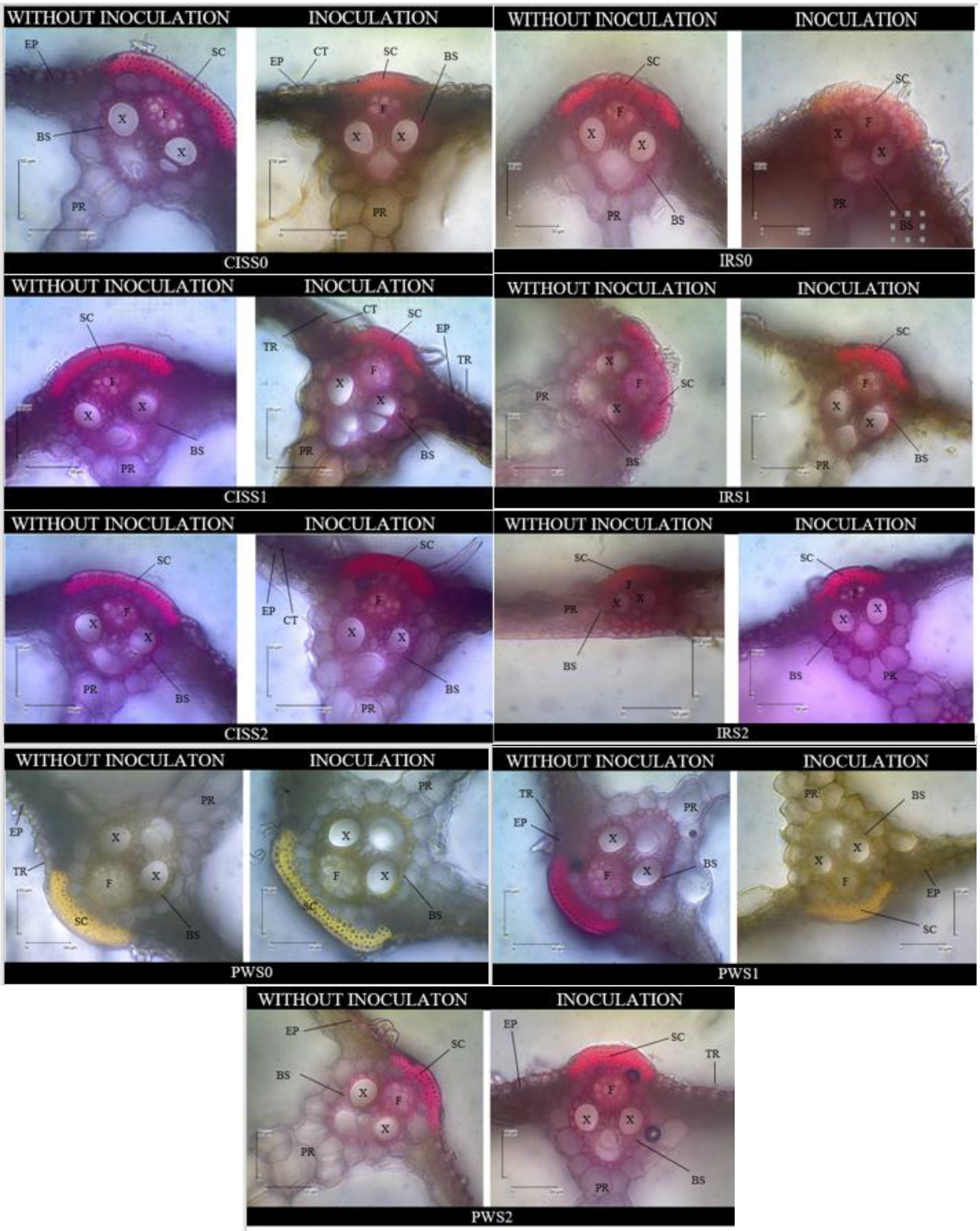

Figure 9. Vascular bundle of IR64 (IR), Cisadane (CIS) and Pandan Wangi (PW) rice cultivar inoculated and non-inoculated *Rhizoctonia solani*. Note: S0: Without silicate fertilizer, S1: 200 kg.ha^{-1} silicate fertilizer, S2: 400 kg.ha^{-1} silicate fertilizer, CT: cuticle, X: Xylem, BS: Bundle sheath, PR: Parenchyma, SC: Sclerenchyma, F: Phloem, EP: Epidermis, TR: Trichome, 40x

Each rice cultivar has diversity in the accumulation of Si elements for high growth and production. The accumulation of Si elements varies widely among different species. Genotypic differences arise due to variations in the Si transporter protein in the roots of rice plants. The accumulated silicon increases biomass, reduces transpiration, increases chlorophyll synthesis, and maintains homeostasis and osmoregulation in cells that affect photosynthesis and plant growth and production (Swain and Rout 2018). Research conducted by Patil et al. (2017) showed that applying calcium silicate fertilizer at a dose of 200 kg ha^{-1} increased the number of panicles. The increased number of panicles was presumably caused by increased photosynthetic activity. The increase in photosynthetic activity was likely caused by an increase in leaf chlorophyll synthesis and nutrient availability due to the application of silicate fertilizers. Thus, silicate fertilizer can increase the synthesis of leaf chlorophyll and indirectly affect the number of panicles due to increased photosynthetic activity in the leaves. Under normal conditions, plants will allocate energy and nutrients for their growth and yield production, but under stress conditions, plants will use much energy and nutrient to survive (Juhaeti et al. 2020).

Several studies have proven that the Si element participates in the metabolic process of the interaction system of pathogens with plant hosts, activates host defense genes through a series of physiological and biochemical reactions as well as signal transduction that induces the expression of resistance in plants to diseases caused by fungi (Song et al. 2016). When a necrotic-causing pathogen infects a plant, the plant develops resistance to further attack by the pathogen, known as systemic acquired resistance (SAR). Silicon application in plants can induce the activity of enzymes such as chitinase (CHI) and peroxidase (POX) and antifungal compounds that can induce a defense response similar to SAR (Sakr 2016b). Schurt et al. (2014) found that the increased activity of phenylalanine ammonia-lyase (PAL), peroxidase (POX), polyphenol oxidase (PPO), and chitinase (CHI) in the leaf midrib of rice plants treated with silicon led to a reduction in the length of the lesions due to the sheath blight of *R. solani*. The enzyme PAL (phenylalanine ammonia-lyase) catalyzes the deamination of L-phenylalanine to produce various phenolic compounds and phytoalexins with lignin as the final product. POX (peroxidase) acts as a defense response by producing antimicrobial hydrogen peroxide, which is involved in cell wall lignification and cell wall proteins (Schurt et al. 2014).

All cultivars have lignified sclerenchyma and bundle vessel, indicated by a red thickened section under the epidermis (Figure 9). Lignification is induced by pathogen penetration and affects host resistance mechanisms, thereby triggering lignin biosynthesis. Penetration of pathogen-increased reactive oxygen species (ROS) and activate mitogen-associated protein (MAP) kinase to induce pathogen-related genes. The anatomically most relevant cell types for pathogen defense are not fully studied (Li et al. 2020). The thicker diameter relates to the plants' growth (Zhang et al. 2006). The induction of Si triggers growth because Si supports the plants' nutrient uptakes. The inoculated plants with a thicker leaf sheath diameter are caused by a suspected defense mechanism against the pathogen by forming a physical barrier. The physical barrier refers to the thickening of sclerenchyma tissue through lignification (Li et al. 2020). Lignification of sclerenchyma cells occurs beneath the epidermis cells. Some of the reasons sclerenchyma cells become targets for defense against invading pathogens are because sclerenchyma cells consist of several layers forming a thicker zone than the epidermis, which consists of a single layer of cells, and the capacity of sclerenchyma cells can be fully lignified to form a strong structure, in addition to parenchymal cell anatomy and bundle vessels are tissues with the most active cellular activity and are nutrient supply areas so that invading pathogens will find it difficult to penetrate and take nutrients from the host because pathogenic hyphae are difficult to penetrate lignified sclerenchyma cells in the carrier bundle (Li et al. 2020).

In conclusion, fertilizer and the severity of the pathogen infection are positively correlated. Silicate fertilization affects the physiological responses of rice to the pathogen *R. solani* which is shown by forming a physical barrier to the pathogen, such as thickening sclerenchyma in inoculated susceptible cultivars. Susceptible cultivars absorb silicate minerals more efficiently, which also improves lignin formation. Silicate fertilizer strengthens plant tissue physiologically in sensitive cultivars Cisadane by increasing lignin concentration, sclerenchyma and cuticle thickness more effectively than IR64 (moderately resistant cultivar) and Pandan Wangi (resistant cultivar).

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