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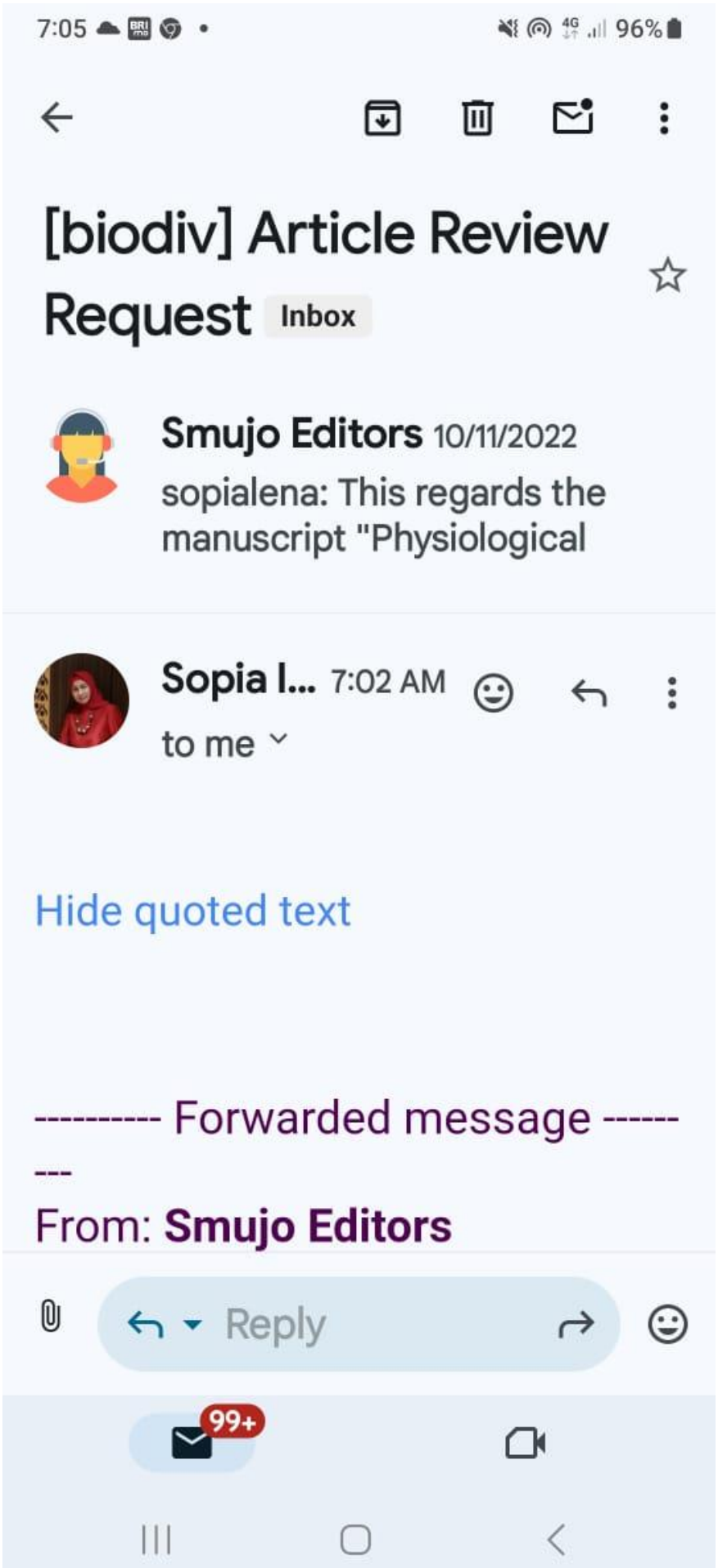
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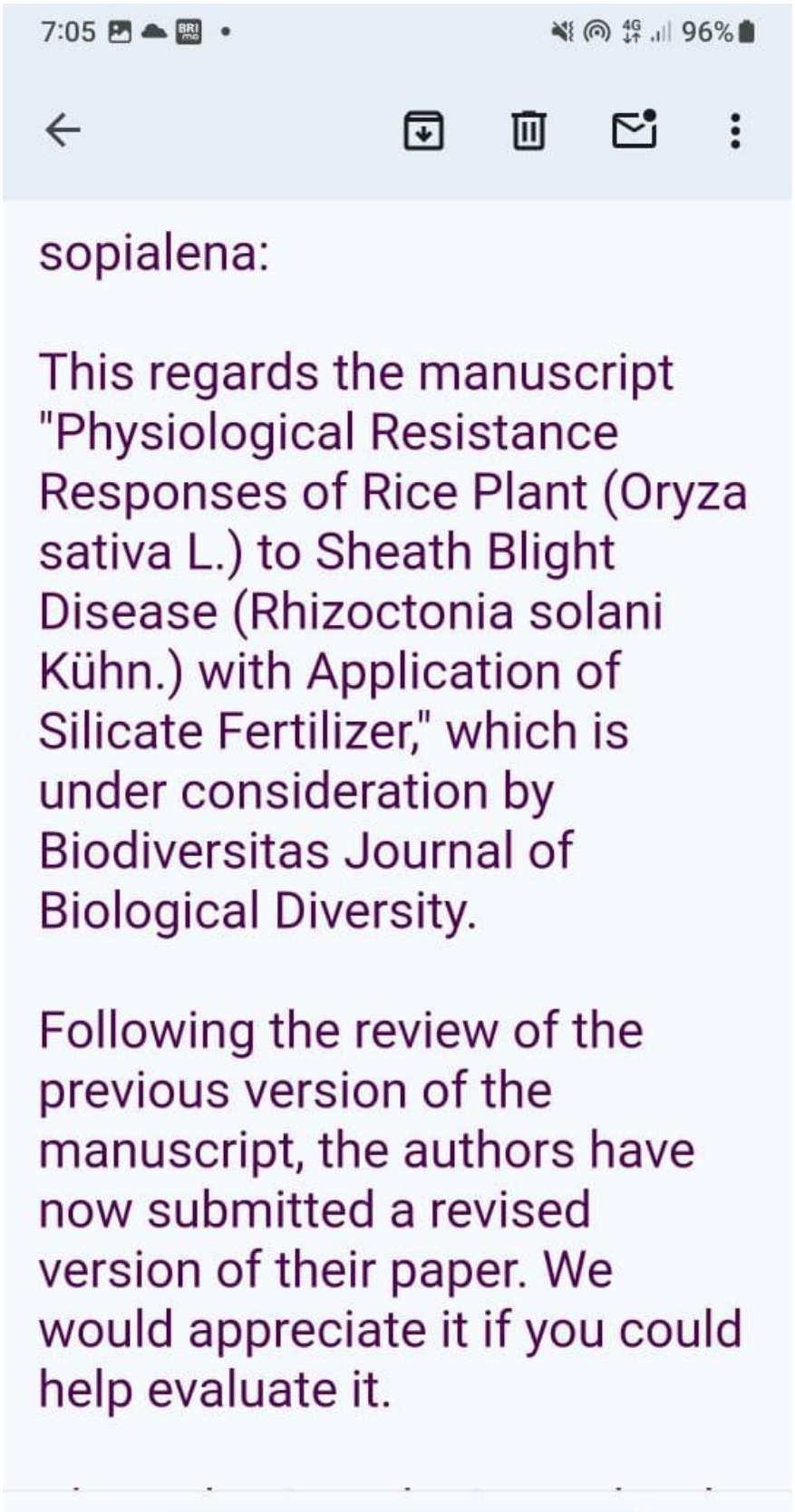
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Following the review of the previous version of the manuscript, the authors have now submitted a revised version of their paper. We would appreciate it if you could help evaluate it.
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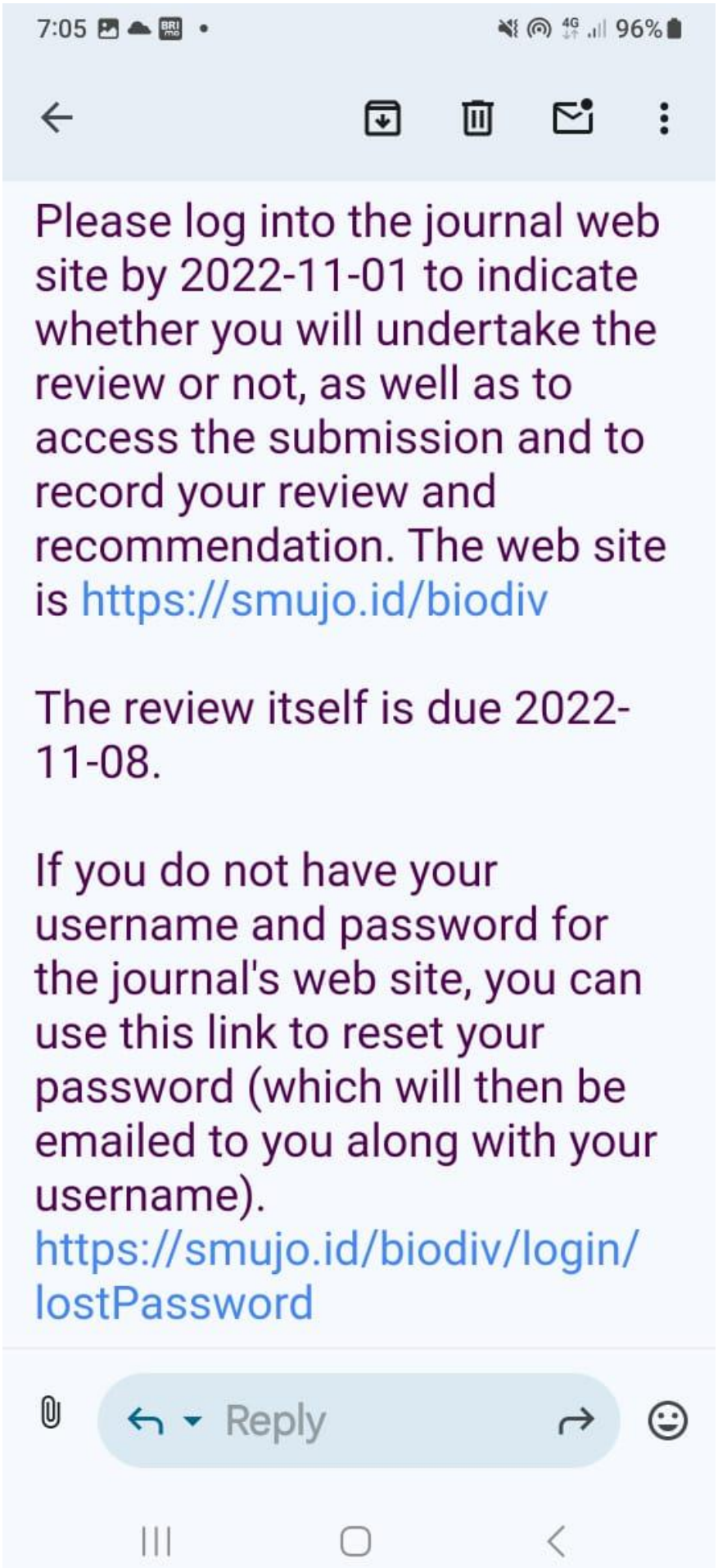
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solani Kuhn generally leads to the reduction of rice yields. The resistance to pathogens can be increased by the application of silicate fertilizers. This research aimed to determine the effect of silicate fertilizer application on the physiological resistance responses structurally and biochemically in rice plants to defend against the rice sheath blight pathogen *Rhizoctonia solani* Kühn. The sample of rice plants consists of Pandan Wangi (PW), Cisadane (CS), and IR64 (IR) which were treated with 3 doses of silicate fertilizer S0 = 0 $kg.ha^{-1}$, S1 = 200 $kg.ha^{-1}$ and S2 = 400 $kg.ha^{-1}$ with inoculation and without inoculation as a control. Relative Lesion Height (RLH)

silicate fertilizer application and cultivar had a significant effect on reducing lesion symptoms. Application of silicate fertilizer and inoculation treatment on different rice cultivars had a significant effect on several parameters such as physiological (chlorophyll and relative water content), growth (plant height and number of leaves), anatomical (leaf sheath diameter and cuticle thickness) and lignin content as a biochemical parameter based on ANOVA and Duncan's test with 95% confidence level. The results showed that PWS1 had the most effect on reducing the percentage of RLH. PWS1 without inoculation

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

Abstract. Sheath blight disease caused by the fungus *Rhizoctonia solani* Kühn generally leads to the reduction of rice yields. The resistance to pathogens can be increased by the application of silicate fertilizers. This research aimed to determine the effect of silicate fertilizer application on the physiological resistance responses structurally and biochemically in rice plants to defend against the rice sheath blight pathogen *Rhizoctonia solani* Kühn. The sample of rice plants consists of Pandan Wangi (PW), Cisadane (CS), and IR64 (IR) which were treated with 3 doses of silicate fertilizer S0 = 0 kg.ha^{-1}, S1 = 200 kg.ha^{-1} and S2 = 400 kg.ha^{-1} with inoculation and without inoculation as a control. Relative Lesion Height (RLH) percentage showed that silicate fertilizer application and cultivar had a significant effect on reducing lesion symptoms. Application of silicate fertilizer and inoculation treatment on different rice cultivars had a significant effect on several parameters such as physiological (chlorophyll and relative water content), growth (plant height and number of leaves), anatomical (leaf sheath diameter and cuticle thickness) and lignin content as a biochemical parameter based on ANOVA and Duncan's test with 95% confidence level. The results showed that PWS1 had the most effect on reducing the percentage of RLH. PWS1 without inoculation had a positive effect on the plant height and leaf sheath diameter. PWS2 without inoculation had the most effect on relative water content. IRS1 without inoculation had the most positive effect on cuticle thickness and lignin content.

Comment [U1]: What is it stand for?

Key words: cultivars, physiological resistance, sheath blight disease, silicate fertilizer

Running title: physiological resistance responses of rice

INTRODUCTION

Rice (*Oryza* sativa) 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 that has abundant rice yields, but the rice yields significantly decrease caused of pests such as pathogens, insect attacks, or weeds (Savary et al., 2000). Sheath blight disease is one of the diseases in rice plants. The disease is caused by the soil-borne fungus *Rhizoctonia solani* Kühn (Yang & Li, 2012). Rice sheath blight is commonly found in various regions in Indonesia, especially in Central Java. According to Indonesian Center for Rice Research (ICRC) (2009), rice sheath blight is found in Indonesia's highland to lowland rice ecosystems.

Rice sheath blight can cause symptoms of lesions in the infected area. Spreading lesions cause rice plant stalks to become 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; Suthin Raj *et al*., 2019). The filling of rice grains that is not optimal result in a drastic decrease in rice plant productivity due to a large number of empty rice grains. Rice sheath blight caused a significant reduction in yields. The decrease in rice yields due to sheath blight disease can reach 30%. The attack of rice sheath blight pathogens can reduce rice yields by up to 50% in susceptible cultivars and

favorable environmental conditions for the development of the disease (Groth, 2008) 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 biochemical (chemical defense). The physiological response is defined structurally by forming a defense barrier in the form of cuticle or cellulose in the epidermal tissue to strengthen the plant's cell wall components (Seal et al., 2018). While the biochemical response (chemical defense) of plants as the plant's defense mechanism, one of which is to produce secondary metabolites in form of lignin (Leroy et al., 2019; Seal et al., 2018) which accumulates in the cell walls of plant tissues. Lignin is closely related to cell rigidity, providing strength and resistance to plant cell walls so that cells become more rigid and can be a barrier that protects plants from pathogenic fungus.

The response of plant resistance to pathogens, especially in rice plants, can be improved by 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 accumulation of silicon in rice plants can increase resistance, increase growth and productivity of rice plants (Seal et al., 2018). Silicon plays an important role in the formation of plant tissue, especially to increase the strength of the tissue by forming a structural barrier in the form of silica-cuticle and silica-cellulose. The structural barrier Si plays an active role in increasing plant resistance to various infectious disease-causing pathogens (Ma & Yamaji, 2006; Seal et al., 2018). Silicon plays a role in inducing the accumulation of lignin and activating the defense mechanism of rice plants from pathogen attacks such as pathogen attack (*Rhizoctonia solani* Kühn.) 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 application silicate fertilizers against infection with rice leaf sheath blight (*Rhizoctonia solani* Kühn.). The plant treated with 3 different factors, namely cultivar, silicate fertilizer dose, and inoculation treatment. The rice cultivars including Pandan Wangi (PW), Cisadane (CS), and IR64 (IR) were given 3 doses of fertilizer, without silicate fertilizer, 200 $kg.ha^{-1}$, and 400 $kg.ha^{-1}$ and inoculation with the fungus *Rhizoctonia solani* Kühn and without as a control. By observing physiological, biochemical and anatomical parameter, the important role of beneficial silicon element to increase plant resistance against leaf sheath blight can be defined.

MATERIALS AND METHODS

Experimental design and treatment

The study was conducted a completely randomized design with silicate fertilizer treatment which consist of 3 doses including S0 = without silicate fertilizer (control), S1 = 200 $kg.ha^{-1}$ and S2 = 400 $kg.ha^{-1}$ of silicate fertilizer ($CaSiO_3$) on 3 different cultivars of Pandan Wangi (PW), Cisadane (CS) and IR64 (IR) that were taken from farmers group of Grabag Purworejo, Jawa Tengah and Indonesian Center for Rice Research. The plants were also divided into 2 groups, the plants that were inoculated and without inoculation of the fungus *Rhizoctonia solani* Kühn with three replications. Mycelia isolates of *Rhizoctonia solani* Kühn were obtained from the Department of Pests and Plant Diseases, Faculty of Agriculture, UGM. Inoculation of the fungus *Rhizoctonia solani* Kühn using the aluminum foil and micro-chamber method based on Jia *et al.* (2013) which was modified at the stage of making *Rhizoctonia solani* Kühn 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 / RWC (Barrs & Weatherley, 1962), and chlorophyll content (Harborne, 1987), anatomical parameters which include the diameter of the leaf sheath, the thickness of the cuticle, the thickness of sclerenchyma tissue and the length 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).

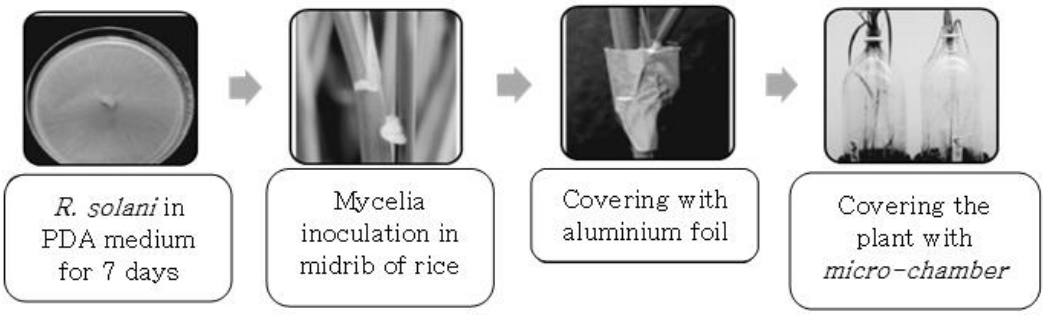

Figure 1. Aluminum Foil Method with Micro-chamber (Jia et al., 2013; Park et al., 2008)

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Procedures

Relative Lesion Height (RLH)

Inoculation treatment was conducted when rice plants' age was 40 day after planting (DAP). After 7 days of sheath blight disease symptoms appeared, the lesion was measured and counted to determine the percentage of blight disease symptoms which were in form of relative lesion height (RLH) percentage. The blight symptoms percentage of RLH was used to count the damage level of pathogen infection by measuring the length of infection area (lesion) and the plants' height using the following formula:

$$\text{RLH (\%)} = \frac{Lesion\ height}{Plant\ height} \text{ x } 100\%$$

Scoring evaluation of RLH (*Relative Lesion Height*):

0 = *Highly resistant* = not infected,

1 = *Resistant* = lesion height is less than 20% from the plant height

3 = *Moderately resistant* = lesion height is between 20-30% of plant height

5 = *Moderately susceptible* = lesion height is between 31-45% of plant height

7 = *Susceptible* = lesion height is between 46-65% of plant height

9 = *highly susceptible* = lesion height is more than 65% of plant height

Relative Water Content (RWC)

The percentage of relative water content in leaves was measured based on method of Barrs & 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), then the sample was immersed in distilled water at 25˚C for 4 hours and reweighed to determine the turgid weight (TW), then the leaf sample was dried in an oven at 80˚C for 24 hours to determine the dry weight (DW). The results of the calculation of FW, TW, and DW then was calculated to find the value of the relative water content in the leaves using the following formula:

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

Keterangan:

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 and 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 3 times. 1 mL of the sample solution was put into the cuvette. The spectrophotometer was calibrated using 80% acetone before use. Each extract measured its absorbance 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:

$$\text{Total of chlorophyll content} = (17{,}3\ A_{646}) + (7{,}18\ A_{663})\ \text{mg.L}^{-1}$$

Lignin Content

Lignin content was measured using 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 length of 3-5 cm. Samples were dried in 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 and 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 10 mL of 72% H2SO4 at a temperature of 47˚C while stirring 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. Cool and let stand until the lignin precipitate settles completely. The cooled sample is then filtered with filter paper whose weight is known to separate the soluble (solution) and insoluble (precipitate) fractions. Wash the lignin precipitate until it is free from acid with hot water. Dry the filter paper containing the lignin precipitate in an oven at 105ºC, cool and weigh to a constant weight (Badan Standardisasi Nasional Indonesia, 2008; Nawawi et al., 2019). Lignin content calculation using the Klason method used the following formula:

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

Comment [U3]: english

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 Analysis

The anatomical cross-section of the midrib was also observed qualitatively using a binocular microscope assisted by an Optilab. Methods to prepare the preparations employed the semi-permanent preparations method. The anatomical parameters including the diameter of the midrib and the thickness of the cuticle of the midrib.were performed using Image Raster software

Data analysis

The data of this study consisted of physiological parameters such as the percentage of sheath blight symptoms, relative water content/RWC, and chlorophyll levels, anatomical parameters such as midrib thickness, cuticle, sclerenchyma thickness, sclerenchyma length and accumulation of lignin content. Qualitative data was presented in descriptive form while quantitative data was analyzed using One Way ANOVA at 95% reliability level and followed by a DMRT test at 5% significance level if there was a significant difference.

Comment [U4]: to see clearly the interaction among 3 level tratments (varieties, inoculation ar non-inoculation, and dose of silicate fertilizer, be if the data analized using the interaction analysis (two way analysis)

RESULTS AND DISCUSSION

RESULTS

Relative Lesion Height

Based on the relative lesion height (RLH percentage (Table 1), the application of silicate fertilizers generally reduced the percentage of RLH in infected rice plants. The highest RLH was found in plants without silicate fertilizer (control). RLH value in Cisadane cultivar without silicate fertilizer of 19.1%, IR 64 cultivar without silicate fertilizer (IRS0) 15.53%, Pandan Wangi cultivar without silicate fertilizer of 13.60%. This means that rice plants without silicate fertilizer showed a higher RLH average value than rice plants with silicate fertilizer, it can be concluded that the application of silicate fertilizers affects the percentage of RLH in rice plants that were infected by the sheath blight pathogen *Rhizoctonia solani* Kühn.

Comparison of RLH value at the treatment of 200 kg.ha^{-1} and 400 kg.ha^{-1} fertilizers doses showed different results for each cultivar. In the Pandan Wangi cultivar, the RLH at a dose of 200 kg.ha^{-1} was 3.33% and the RLH at a dose of 400 kg.ha^{-1} was 6.06%. Pandan Wangi cultivar showed that the RLH value of plants treated with a dose of 200 kg.ha^{-1} was lower than that of a fertilizer treated with a dose of 400 kg.ha^{-1}. This indicates that the application with a dose of 200 kg.ha^{-1}of silicate fertilizer can increase the resistance of rice plants to the Pandan Wangi cultivar. Meanwhile, the IR64 cultivar showed RLH at a dose of 200 kg.ha^{-1} of 7.067% and a dose of 400 kg.ha^{-1} of 5.73%. Cisadane cultivar with a dose of 200 kg.ha^{-1} showed an RLH value of 8.46% and RLH at a dose of 400 kg.ha^{-1} of 5.90%. Cisadane and IR64 cultivars showed a different pattern of RLH values from Pandan Wangi. Cisadane and IR64 cultivars, the RLH value for fertilizer application at a dose of 400 kg.ha^{-1} was lower than the average RLH for fertilizer at a dose of 200 kg.ha^{-1}. This means that the application of silicate fertilizer at a dose of 400 kg.ha^{-1} has a more positive effect on the resistance of Cisadane and IR64 cultivars. Pandan Wangi cultivar with fertilizer at a dose of 200 kg.ha^{-1} (PWS1) was the most influential treatment on the percentage of RLH based on Duncan's test results with a 95% reliability level (Table 1).

Table 1. Physiological data on Pandan Wangi, Cisadane and IR64rice cultivars at 48 DAP with silicate fertilizer and *Rhizoctonia solani* Kühn Inoculation

Characters	Pandan Wangi						Cisadane						IR64					
	Inoculation			Without inoculation			Inoculation			Without inoculation			Inoculation			Without inoculation		
	S0	S1	S2	S0	S1	S2	S0	S1	S2	S0	S1	S2	S0	S1	S2	S0	S1	S2
Relative Lesion Height (%)	13.60^{d}	3.33^{a}	6.06^{b}	-	-	-	19.10^{e}	8.46^{c}	5.90^{b}	-	-	-	15.53^{d}	7.067^{bc}	5.73^{b}	-	-	-
Chlorophyll Content (mg/L^{-1})	6.275^{abc}	7.626^{c}	8.694^{cd}	12.897^{ef}	18.114^{ij}	15.096^{fgh}	3.557^{a}	6.081^{abc}	7.054^{bc}	13.499^{efg}	21.312^{k}	14.058^{efg}	4.375^{ab}	8.112^{c}	11.159^{de}	4.375^{ab}	8.112^{c}	11.159^{de}
Relative Water Content (%)	65.33^{a}	84.26^{c}	75.36^{b}	81.10^{c}	85.63^{c}	94.86^{d}	66.13^{a}	85.50^{c}	75.93^{b}	74.86^{b}	84.70^{c}	82.50^{c}	65.30^{a}	82.00^{c}	81.86^{c}	66.80^{a}	82.63^{c}	81.93^{c}
Lignin Content (%)	14.73^{ef}	13.04^{a}	14.75^{b}	13.72^{abcd}	18.83^{k}	14.30^{ef}	14.24^{cdef}	13.51^{ab}	15.56^{h}	15.44^{gh}	14.15^{bcde}	13.71^{abcd}	14.89^{fg}	18.69^{k}	16.70^{i}	13.59^{abc}	17.92^{j}	16.75^{f}

Note:The number followed by the same letter in the same row has no significant difference based on DMRT α = 0.05 n = 3

Comment [U5]: the data only presented in or way, then we can not see clearly the different among the varieties

Chlorophyll Content

In general, the result showed that the lowest chlorophyll was found in plants without silicate fertilizer in the three cultivars, while the chlorophyll content in plants with a dose of 200 kg.ha^{-1}and a dose of 400 kg.ha^{-1}revealed variations. In the inoculation treatment, the three cultivars had the same pattern. The chlorophyll content in the silicate fertilizer treatment at a dose of 200 kg.ha^{-1} was higher than a dose of 400 kg.ha^{-1}. In Pandan Wangi inoculation cultivar, plants with a dose of 400 kg.ha^{-1} of fertilizer had a higher chlorophyll content at the value 8.694 mg.L^{-1} compared to the treatment of 200 kg.ha^{-1} of fertilizer which was only 7.626 mg.L^{-1}. In the inoculated Cisadane cultivar, plants with a dose of 400 kg.ha^{-1} silicate fertilizer also had a higher chlorophyll content value 7.054 mg.L^{-1}compared to fertilizer treatment at a dose of 200 kg.ha^{-1} of 6.081 mg.L^{-1}. In IR64 cultivar, inoculated plant with fertilizer treatment at a dose of 400 kg.ha^{-1} had a higher chlorophyll content value of 11.159 mg L^{-1} and chlorophyll content at a dose of 200 kg.ha^{-1} fertilizer treatment was only 8.112 mg.L^{-1}.

In the condition without inoculation, plants responded differently to the application of silicate fertilizer depending on cultivar factors. In the Pandan Wangi cultivar without inoculation, the chlorophyll content of plants treated with a dose of 200 kg.ha^{-1} was higher than that of a silicate fertilizer at a dose of 400 kg.ha^{-1}, a value of 18.114 > 15.096 mg.L^{-1}. The Cisadane (CS) cultivar without inoculation also had the same pattern as Pandan Wangi, the chlorophyll content of plants treated with silicate fertilizers at a dose of 200 kg.ha^{-1} was higher than those treated with silicate fertilizers at a dose of 400 kg.ha^{-1} a value of 21.312 > 14.058 mg.L^{-1}. While the rice cultivar IR 64 showed a different pattern, in fact the chlorophyll content in plants treated with silicate fertilizers at a dose of 200 kg.ha^{-1} was lower than those treated with silicate fertilizers at a dose of 400 kg.ha^{-1}, a value of 17.822 < 19.433 mg.L^{-1} (Table 1, Figure 1).

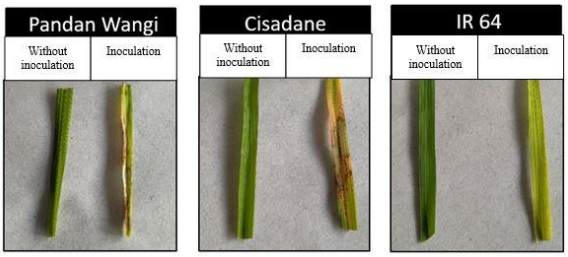

Figure 1. Differences in rice plants' leaf color of Pandan Wangi, Cisadane, and IR64 cultivars with silicate fertilizer without inoculation and inoculation of the sheath blight pathogen *Rhizoctonia solani* Kühn.

Relative Water Content

All the control plants without silicate fertilizer application with *Rhizoctonia solani* Kühn inoculation had relative water content in the range of 60-70%, divided into PWS0 inoculation (65.33%), CSS0 inoculation (66.13%), and IRS0 inoculation (65.30%). The majority of control plants without silicate fertilizer (S0) had lower relative water content than plants with silicate fertilizer, except for PWS0 plants without inoculation had RWC of 81.10%. Even IRS0 plants without inoculation had relative water content of 66.80% that showed signs of early wilting of plants (Table 1).

Lignin Content

The lignin content in Pandan Wangi cultivar ranged from 13.04% to 18.83% with the highest lignin content in PWS1 plants without inoculation was 18.83% and the lowest lignin content in inoculated PWS1 plants was 13.04% (Table 1). PWS2 inoculated plants had more lignin content than inoculated PWS1 which was 14.75% > 13.04% while for plants without inoculation, the plants with a fertilizer dose of 200 kg.ha^{-1} (PWS2 without inoculation) had more lignin content than plants with fertilizer dose of 400 kg.ha^{-1} (PWS2 without inoculation) at the value of 14.30%. This showed that silicate fertilizer at a dose of 400 kg.ha^{-1} was more influential in increasing lignin level in the inoculation treatment, whereas silicate fertilizer at a dose of 200 kg.ha^{-1} was more influential in increasing lignin level in the control treatment without inoculation.

The lignin content of the Cisadane cultivar (CS) was in the range of 13.51 – 15.56% with the highest lignin content in plants with 400 kg.ha^{-1} (CSS2) fertilizer with inoculation (15.56%) and the lowest lignin content in plants with the application of 200 kg.ha^{-1} (CSS1) fertilizer inoculation (13.52%). Application of silicate fertilizer of 400 kg.ha^{-1} had significantly increased lignin content in inoculated plants, whereas plants without inoculation with a dose of 200 kg.ha^{-1} of silicate fertilizer had higher lignin content than plants with 400 kg.ha^{-1} of silicate fertilizer. This indicates that the inoculation of plants with 400 kg.ha^{-1} of silicate fertilizer was more influential in increasing lignin levels, while plants without inoculation treatment showed the opposite effect, plants with a dose of 200 kg.ha^{-1} of fertilizer had more effect on increasing lignin levels.

The lignin content of IR64 plants was in the range of 13.59% to 18.69% with the highest lignin levels found in inoculated IRS1 plants was 18.69% and the lowest lignin levels in IRS0 without inoculations was 13.59%. Plant treated with *R.solani* inoculation and a dose of 200 kg.ha^{-1} of silicate fertilizer had a higher lignin content than with a dose of 400 kg.ha^{-1}, while the plants without inoculation also had the same pattern, plants with a dose of 200 kg.ha^{-1} of silicate fertilizer were higher than with 400 kg.ha^{-1}. This demonstrated that in rice cultivar of IR64 (IR) with or without inoculation, the silicate fertilizer application at a dose of 200 kg.ha^{-1} has more effect on increasing lignin levels (Table 1).

Anatomical Analysis

In terms of fertilizer dose, the control plants without silicate fertilizer had midrib diameter ranged from 955.98 µm to 1254.82 µm, plants with 200 kg.ha^{-1} silicate fertilizer had midrib diameter ranged from 1033.19 µm to

1573.03 µm, and plants with silicate fertilizer a dose of 400 kg.ha^{-1} has a midrib diameter in the range of 1046.25 – 1360.38 µm. Based on these data, it is known that in general, the plants with silicate fertilizer have midrib that tend to be thicker than control plants without silicate fertilizer application. In all cultivars, the average diameter of the midrib with fertilizer treatment at a dose of 400 kg.ha^{-1} was smaller than that at a dose of 200 kg.ha^{-1}. This indicated that the silicate fertilizer at a dose of 200 kg.ha^{-1} has a more positive effect on the thickness of the midrib diameter. In Pandan Wangi cultivar, the midrib diameter without inoculation was thicker than in inoculated. Application of silicate fertilizer of 200 kg.ha^{-1} plants without inoculation had the largest midrib diameter. Plants without inoculation treatment had thicker diameters than inoculated plants, especially the Pandan Wangi cultivar. However, the Cisadane and IR64 cultivars have the opposite pattern. In the Cisadane cultivar, the inoculated with silicate fertilizer 200 kg.ha^{-1} had larger midrib diameter than the non-inoculated and in the IR64 cultivar, the inoculated with silicate fertilizer 200 kg.ha^{-1} also had a larger diameter than plant without inoculation (1033.19 µm) as can be seen in Table 2.

In general, the cuticle thickness of control plants without silicate fertilizer was in the range from 3.19 µm to 3.72 µm. 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. Based on these data, it can be concluded that plant treated with silicate fertilizer treatment had a thicker cuticle thickness than the control plants (Table 2, Figure 2).

All cultivars have sclerenchyma areas under the epidermis but the thickness of sclerenchyma is different in each cultivar. Cisadane cultivars without inoculation had a more elongated sclerenchyma tissue in the range of 144.98 – 155.24 µm compared to inoculated plants. In inoculated Cisadane plants, sclerenchyma tissue was shorter in the length ranged from 84.73 µm to 114.75 µm. Although it looked shorter, the tissue was thicker with a thickness of 17.43 – 24.18 µm compared to sclerenchyma tissue of Cisadane without inoculation. In the IR64 cultivar, it was seen that the sclerenchyma area of plants without inoculation had a more elongated area in the range of 89.01 µm to 155.98 µm compared to plant with inoculation treatment which was only in the range of 53.56 – 137.65 µm but inoculated plants have thickened sclerenchyma tissue in the range of 11.48 µm to 24.18 µm. Based on these data, it can be concluded that the Cisadane and IR64 cultivars in the inoculation treatment had shorter and thicker sclerenchyma tissue, while in the treatment without inoculation the sclerenchyma tissue was longer but the thickening was not as visible. In the Pandan Wangi (PW) cultivar, the inoculated plants had a longer sclerenchyma tissue area in the range of 100.03 µm to 181.70 µm compared to plants without inoculation which only had a sclerenchyma length range of 98.58 µm to 121.92 µm (Table 2, Figure 4).

Table 2. Anatomical data on Pandan Wangi, Cisadane and IR64 rice cultivars at 48 DAP with slicate fertilizer and *Rhizoctonia solani Kühn* Inoculation

Characters	Pandan Wangi						Cisadane						IR64					
	Inoculation			Without inoculation			Inoculation			Without inoculation			Inoculation			Without inoculation		
	S0	S1	S2	S0	S1	S2	S0	S1	S2	S0	S1	S2	S0	S1	S2	S0	S1	S2
Sheath diameter (µm)	1229.04^{fg}	1525.10^{k}	1360.38^{j}	1222.27^{f}	1573.03^{l}	1325.23^{i}	1254.82^{gh}	1330.91^{i}	1274.65^{h}	1097.97^{d}	1263.93^{h}	1060.10^{bc}	955.98^{a}	1193.57^{e}	1046.25^{bc}	1058.41^{bc}	1033.19^{b}	1072.15^{cd}
Cuticle thickness (µm)	3.72^{cd}	4.96^{h}	4.50^{f}	3.22^{ab}	4.61^{fg}	3.72^{cd}	3.21^{a}	4.75^{fgh}	3.78^{d}	3.22^{ab}	4.73^{fgh}	3.53^{bcd}	3.19^{a}	4.86^{gh}	3.42^{abc}	3.54^{cd}	4.17^{e}	4.73^{fgh}
Sclerenchyma length (µm)	181.70^{i}	104.14^{cde}	100.03^{bcde}	121.92^{f}	115.75^{ef}	98.58^{bcd}	84.73^{b}	98.98^{bcd}	114.75^{def}	155.24^{h}	152.04^{gh}	144.98^{gh}	137.65^{g}	96.63^{bc}	53.56^{a}	155.98^{h}	151.69^{gh}	89.01^{bc}
Sclerenchyma thickness (µm)	17.19^{bc}	19.32^{de}	24.20^{h}	21.14^{f}	24.12^{h}	19.40^{de}	17.43^{bc}	24.18^{h}	22.21^{g}	18.60^{d}	20.59^{f}	16.93^{b}	19.06^{de}	24.18^{h}	11.48^{a}	19.80^{e}	19.30^{de}	17.84^{c}

The number followed by the same letter in the same row has no significant difference based on DMRT $\alpha = 0.05$ n = 3

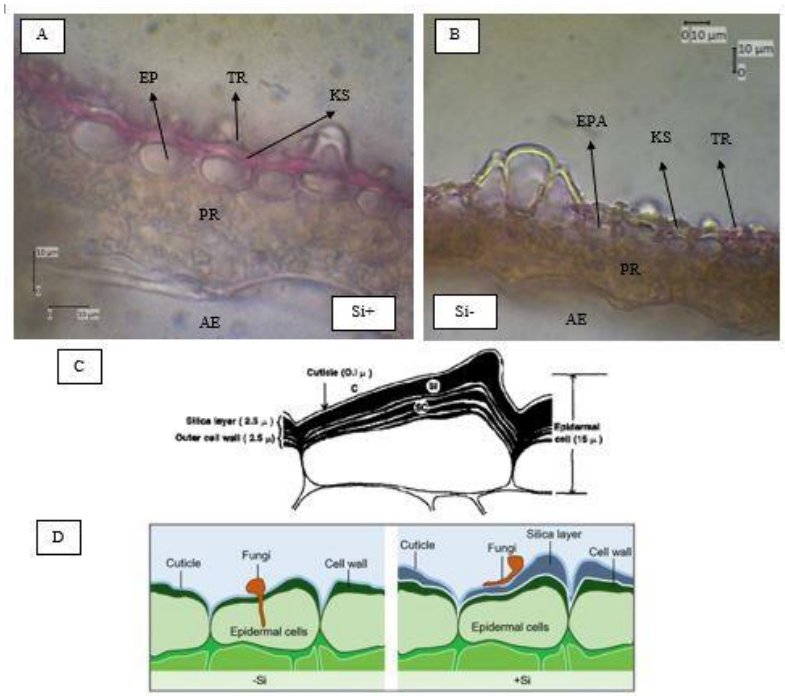

Figure 2. Epidermis and cuticle-silica cell layers in plants with silicate fertilizer (A) and epidermal layers of plants without silicate fertilization (B) Cuticle-silica double layers on leaf blades (Rao et al., 2015) (C) Illustration Schematic Si- and Si+ Epidermis Layer (M. Wang et al., 2017) (D) (EP = Epidermis, TR = Trichome, KS = Cuticle-Silica, PR = Parenchyma, AE = Aerenchym)

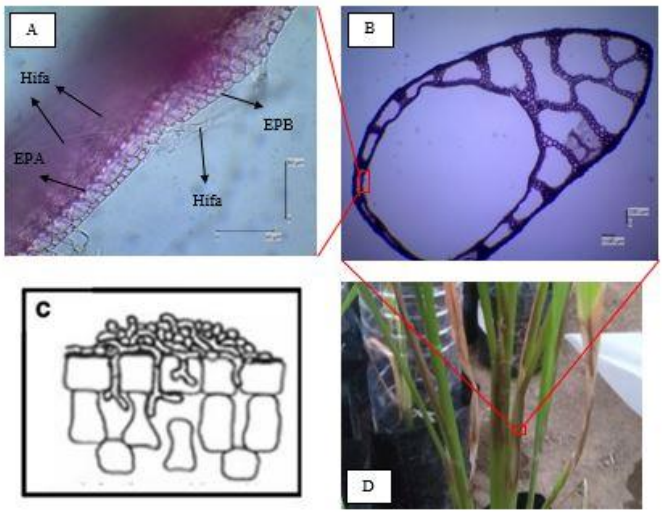

Figure 3. The Damaged epidermal cell layer by pathogens (A) Cross section of rice sheath (B), *R. solani* fungal penetration schematic (C) (Taheri & Tarighi, 2011), Lesion on infected rice sheath (D) (EPA = Upper Epidermis, EPB = Lower Epidermis)

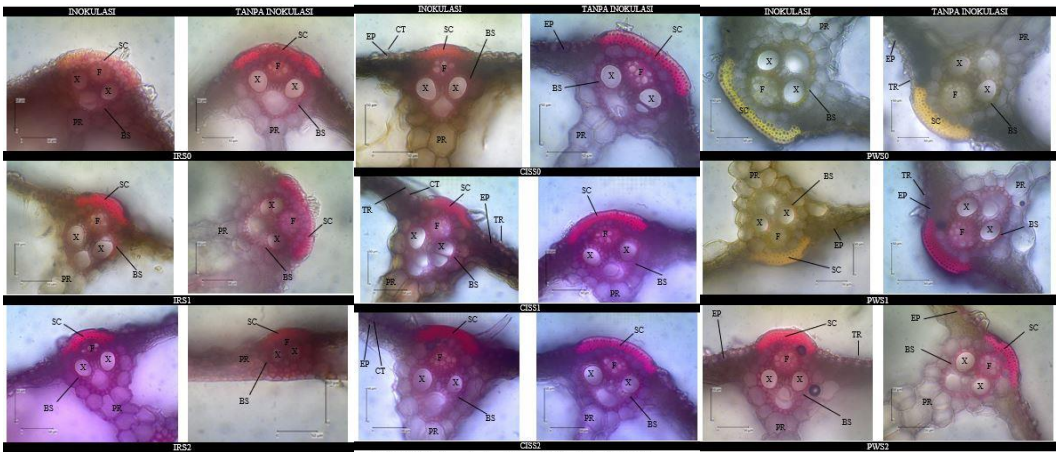

Figure 4. Vascular bundle of IR64 (IR), Cisadane (CIS) and Pandan Wangi (PW) rice cultivar inoculated and non-inoculated *Rhizoctonia solani Kühn* (CT = cuticle, X = Xylem, BS = Bundle sheath, PR = parenchyma, SC = sclerenchyma, F = phloem, EP = epidermis, TR = Trichome, 40x)

Discussion

The infection of the sheath blight pathogen caused by *Rhizoctonia solan*i Kühn shows symptoms in the form lesions. Lesions are necrotic or chlorotic wet sores localized in infected area with the fungus *R. solani.* Lesions appear due to the pathogen *R. solani* have morphological characteristics, they are greenish grey in the infected area. If the environmental conditions are favorable for the pathogen, the lesion will quickly spread and infect the host (Senapati et al., 2022). Necrotic injury is one of the plant defense mechanisms in form of programmed cell death (PCD) which is relatively fast in response to pathogen invasion (Minina et al., 2013)). The presence of lesions on the sheath and leaf blade cause rotting and drying of the entire leaf. Drying of the entire leaf causes the color of the leaves to become more yellow and eventually the leaves prior to early senescence. The yellow color of the leaves occurs due to chlorophyll fluorescence or chlorophyll degradation in areas infected with pathogens. Chlorophyll degradation is used as an indicator of photosynthesis regulation and plant response to stress (Lin et al., 2018). The presence of chlorophyll degradation causes chlorophyll content in plants with inoculation to be lower than without inoculation.

The appeared symptoms of the lesions indicate the severity of the pathogen infection as indicated by the RLH value. The higher the percentage of RLH, the more severe the fungal infection of rice sheath blight. The severity of the infection indicates that the plant is more susceptible to pathogen infection. On the other hand, the lower RLH percentage value, the fewer symptoms of lesions appear and the plant is more resistant to infection with sheath blight pathogens. Based on these data, it can be seen that the Cisadane cultivar is the cultivar that is most susceptible to sheath blight infection because it has the highest RLH value in control and the Pandan Wangi cultivar is the cultivar that is more resistant to sheath blight infection because it has the lowest RLH value in control treatment. Different cultivars showed different RLH values. The resistance of rice plants to biotic stress is also determined by the type of cultivar. Each cultivar provides a fundamental resistance response to the presence of pathogens. Cisadane is resistant to leafhoppers, but is not resistant to midrib blight. According to Suprihatno et al., (2010) and the Indonesian Center for Rice Research, Agricultural Research and Development Agency, and the Ministry of Agriculture, Cisadane cultivar is considered a cultivar that is susceptible to sheath blight pathogens. The IR64 cultivar has high potential in addition to being resistant to brown leafhoppers, it is also relatively resistant to leaf blight, while the Pandan Wangi is a superior cultivar that is resistant to sheath blight pathogens (Sitaresmi *et al.*, 2013). According to research conducted by Sari (2019), it was shown that sheath blight relatively attacks the Pandan Wangi cultivar at an intensity of disease severity below 30%. Breeding rice cultivars that are tolerant to blight is a feasible control measure, but the resistance of

cultivars with 1 or 2 main resistance genes is unstable under field conditions due to high pathogen variability and rapid pathogen evolution (Song et al., 2016) so a combination of external factors is required to increase the plant resistance through silicate fertilizer applications.

The application of silicate fertilizer affects increasing plant resistance to pathogens. Solid silicate fertilizer in the form of calcium silicate ($CaSiO_3$) as fertilizer applied to the soil has a positive effect on reducing the severity of plant diseases caused by pathogens such as bacteria, viruses, and fungi in monocot and dicot plants. In general, the effect of silicon on plant resistance is due to silicon deposition in the cell wall area, acts as a physical defense that it is difficult for pathogens to penetrate and infect plants (Sakr, 2016). 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 GaoFeng *et al.*, (2010) regarding the severity of rice plants given the application of silicon was also reduced to 11.83 - 52.12%.

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 element is absorbed from the soil in the form of monosilicate acid which is dissolved in water, the absorbed water is lost through the transpiration mechanism while silicon remains in plant tissues. Monosilicate acid polymerized into silica gel through non-enzymatic reactions. This polymerization of silicon is commonly identified as silica gel. 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 strength, rigidity, and cell rigidity, minimize transpiration and protect plants from diseases and insects (Rao et al., 2015; Sathe et al., 2021; M. Wang et al., 2017) (Figure 2). Histological studies on infection and symptom development of *R.solani* in rice plants were first investigated by Marshall dan Rush (1980). Pathogens can persist in the soil in the form of sclerotia or soil debris for long periods, when environmental conditions are favorable, sclerotia can germinate and form mycelia. If the mycelia attach to the plant surface, the mycelia will develop and infect the plant by penetrating directly into the plant tissue (Taheri & Tarighi, 2011) as displayed in Figure 3. The inoculated plants become thicker, presumably due to a defense mechanism against pathogens by forming a physical barrier. The physical barrier in question 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 et al., 2015; Sathe et al., 2021; M. Wang et al., 2017) Both physical barriers have the same goal in preventing the penetration of pathogenic fungi.

Rice plants absorb Si in a fertilizer dose range of 230 - 470 $kg.ha^{-1}$ or about 2 times higher than nitrogen uptake (Rao et al., 2015; Subiksa, 2018). The element of Si also plays a role in increasing the availability of nutrients including nitrogen elements (Rao et al., 2015). Nitrogen is one of the essential nutrients for growth and development as well as the main component of chlorophyll and protein which is closely related to leaf color in agricultural plants such as rice plants (Y. 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., 2015; Yoshida, 1981). Leaf stiffness is related to the relative water content of the plant. Normal relative water content values ranged from 98% on leaves that experienced full turgidity, and relative water content values ranging from 60-70% showed early wilting while relative water content values ranged from 30-40% indicated very dry and dying leaves depending on the plant species. (Barrs & Weatherley, 1962). 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 (Wang et al., 2017).

Each rice cultivar has diversity in terms of the accumulation of Si element for high growth and production. The accumulation of Si element varies widely among different species. Genotypic differences that arise due to variations in the Si transporter protein in the roots of rice plants. The accumulated silicon results in an increase in biomass, reduces transpiration, increases chlorophyll synthesis, and maintains homeostasis and osmoregulation in cells that affect photosynthesis and plant growth and production (Swain & Rout, 2018). Research conducted by Patil et al. (2017) showed that the application of calcium silicate fertilizer at a dose of 200 kg/ha was increased the number of panicles. An increase in the number of panicles due to increased photosynthetic activity with an increase leaf chlorophyll synthesis and nutrient availability due to the application of silicate fertilizers. It can be concluded that the application of silicate fertilizer can increase the synthesis of leaf chlorophyll and indirectly affect the number of panicles due to an increase in photosynthetic activity in the leaves.

Several studies have proven that the element Si 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 plant is infected by a necrotic-causing pathogen, 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, 2016). 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 *Rhizoctonia solani* Kuhn. 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 red thickened section under the epidermis (Figure 4). 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 gene. 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 the growth because Si support the plants' nutrient uptakes. The inoculated plants that have 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 the lignification process (Li et al., 2020). Lignification of sclerenchyma cells under the epidermis. 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).

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REFERENCES

Badan Standardisasi Nasional Indonesia. (2008). *Pulp dan kayu - Cara uji kadar lignin - Metode Klason*.

Barrs, H., & Weatherley, P. (1962). A Re-Examination of the Relative Turgidity Technique for Estimating Water Deficits in Leaves. *Australian Journal of Biological Sciences*, *3*(15), 413–428.

Chang, S., Tzeng, D., & Li, C. (2002). Effect of silicon nutrient on bacterial blight resistance of rice (Oryza sativa L.). *Proceedings of the Second Silicon in Agriculture Conference, Tsuruoka, Yamagata, Japan*, 31–33.

GaoFeng, X., WanChun, S., ALin, S., ZhaoJun, L., FenLiang, F., & YongChao, L. (2010). Influence of Silicon On Rice Growth, Resistance to Bacterial Blight and Activity of Pathogenesis-Related Proteins. *Scientia Agricultura Sinica*, *43*(4), 690–697.

Groth, D. E. (2008). Effects of cultivar resistance and single fungicide application on rice sheath blight, yield, and quality. *Crop Protection*, *27*(7), 1125–1130. https://doi.org/10.1016/j.cropro.2008.01.010

Harborne, J. B. (1987). *Metode Fitokimia : Penuntun Cara Modern Menganalisis Tumbuhan* (S. Niksolihin (ed.); Kedua). Penerbit ITB.

IRRI. (2002). *Standard Evaluation System of Rice*.

Jia, Y., Liu, G., Park, D. S., & Yang, Y. (2013). Inoculation and Scoring Methods For Rice Sheath Blight Disease. *Methods in Molecular Biology*, *956*, 257–268. https://doi.org/10.1007/978-1-62703-194-3_19

Leroy, N., Tombeur, F. De, Walgra, Y., & Corn, J. (2019). Silicon and Plant Natural Defenses against Insect Pests : Impact on Plant Volatile Organic Compounds and Cascade E ff ects on Multitrophic Interactions. *Plants*, *8*(11), 444.

Li, W., Wang, K., Chern, M., Liu, Y., Zhu, Z., Liu, J., Zhu, X., Yin, J., Ran, L., Xiong, J., He, K., Xu, L., He, M., Wang, J., Liu, J., Bi, Y., Qing, H., Li, M., Hu, K., … Chen, X. (2020). Sclerenchyma Cell Thickening Through Enhanced Lignification Induced by OsMYB30 Prevents Fungal Penetration of Rice Leaves. *New Phytologist*, *226*(6), 1850–1863. https://doi.org/10.1111/nph.16505

Lin, W., Guo, X., Pan, X., & Li, Z. (2018). Chlorophyll composition, chlorophyll fluorescence, and grain yield change in esl mutant rice. *International Journal of Molecular Sciences*, *19*(10), 1–12. https://doi.org/10.3390/ijms19102945

Ma, J. F., & Yamaji, N. (2006). Silicon Uptake and Accumulation in Higher Plants. *Trends in Plant Science*, *11*(8), 392–397. https://doi.org/10.1016/j.tplants.2006.06.007

Marshall, D. S., & Rush, M. C. (1980). Infection Cushion Formation on Rice Sheaths by Rhizoctonia solani [Histological Aspects of Cultivar esistance]. *Phyto-pathology*, *70*(10), 947–950.

Minina, E. A., Filonova, L. H., Daniel, G., & Bozhkov, P. V. (2013). Detection and Measurement of Necrosis in Plants. *Methods in Molecular Biology*, *1004*, 229–248. https://doi.org/10.1007/978-1-62703-383-1_17

Moreira-Vilar, F. C., Siqueira-Soares, R. D. C., Finger-Teixeira, A., De Oliveira, D. M., Ferro, A. P., Da Rocha, G. J., Ferrarese, M. D. L. L., Dos Santos, W. D., & Ferrarese-Filho, O. (2014). The Acetyl Bromide Method is Faster, Simpler and Presents Best Recovery of Lignin in Different Herbaceous Tissues than Klason and Thioglycolic Acid Methods. *PLoS ONE*, *9*(10), 1–7. https://doi.org/10.1371/journal.pone.0110000

Nawawi, D. S., Sari, R. K., Wistara, N. J., Fatrawana, A., Astuti, P., & Syafii, W. (2019). Karakteristik Lignin Empat Jenis Bambu (Lignin Characteristic of Four Bamboo Species). *Jurnal Ilmu Terknol. Kayu Tropisropis*, *17*(1), 1–7.

Nuryanto, B. (2018). Penyakit Hawar Pelepah (Rhizoctonia solani) pada Padi dan Taktik Pengelolaannya. *Jurnal Perlindungan Tanaman Indonesia*, *21*(2), 63. https://doi.org/10.22146/jpti.22494

Park, D., Sayler, R. J., Hong, Y. G., Nam, M. H., & Yang, Y. (2008). A Method for Inoculation and Evaluation of Rice Sheath Blight Disease. *Plant Disease*, *92*(1), 25–29.

Patil, A., Durgude, A., Pharande, A., Kadlag, A., & Nimbalkar, C. (2017). Effect of Calcium Silicate as a Silicon Source on Growth and Yield of Rice Plants. *International Journal of Chemical Studies*, *5*(6), 545–549.

Rao, G. B., Pi, P. Y., & Syriac, E. K. (2015). Silicon Uptake , Transportation and Accumulation in Rice. *Agricultural Reviews*, *26*(3), 223–228.

Sakr, N. (2016a). Silicon Control of Bacterial and Viral Diseases in Plants. *Journal of Plant Protection Research*, *56*(4), 331–336.

https://doi.org/10.1515/jppr-2016-0052

Sakr, N. (2016b). The role of silicon (Si) in increasing plant resistance against fungal diseases. *Hellenic Plant Protection Journal*, *9*(1), 1–15. https://doi.org/10.1515/hppj-2016-0001

Sathe, A. P., Kumar, A., Mandlik, R., Raturi, G., Yadav, H., Raj, T., & Sonah, H. (2021). Role of Silicon in Elevating Resistance Against Sheath Blight and Blast Diseases in Rice (Oryza sativa L .). *Plant Physiology and Biochemistry*, *166*(March), 128–139. https://doi.org/10.1016/j.plaphy.2021.05.045

Savary, S., Willocquet, L., Elazegui, F. A., Castilla, N. P., & Teng, P. S. (2000). Rice pest constraints in tropical Asia: Quantification of yield losses due to rice pests in a range of production situations. *Plant Disease*, *84*(3), 357–369. https://doi.org/10.1094/PDIS.2000.84.3.357

Schurt, D. A., Cruz, M. F. A., Nascimento, K. J. T., Filippi, M. C. C., & Rodrigues, F. A. (2014). Silicon Potentiates The Activities of Defense Enzymes in The Leaf Sheaths of Rice Plants Infected by Rhizoctonia solani. *Tropical Plant Pathology*, *39*(6), 457–463. https://doi.org/10.1590/S1982-56762014000600007

Seal, P., Das, P., & Biswas, A. K. (2018). Versatile Potentiality of Silicon in Mitigation of Biotic and Abiotic Stresses in Plants: A Review. *American Journal of Plant Sciences*, *09*(07), 1433–1454. https://doi.org/10.4236/ajps.2018.97105

Senapati, M., Tiwari, A., Sharma, N., & Chandra, P. (2022). Rhizoctonia solani Kühn Pathophysiology : Status and Prospects of Sheath Blight Disease Management in Rice Morphological Diversity Based on. *Frontiers in Plant Science*, *13*(May), 1–22. https://doi.org/10.3389/fpls.2022.881116

Sitaresmi, T., Wening, R. H., Rakhmi, A. T., Yunani, N., & Susanto, U. (2013). Pemanfaatan Plasma Nutfah Padi Varietas Lokal dalam Perakitan Varietas Unggul. *Iptek Tanaman Pangan*, *8*(1), 22–30.

Song, A., Xue, G., Cui, P., Fan, F., Liu, H., Yin, C., Sun, W., & Liang, Y. (2016). The Role of Silicon in Enhancing Resistance to Bacterial Blight of Hydroponic- and Soil-Cultured Rice. *Scientific Reports*, *6*(November 2015), 1–13. https://doi.org/10.1038/srep24640

Subiksa, I. G. M. (2018). Pengaruh Pupuk Silika terhadap Pertumbuhan dan Hasil Tanaman Padi Sawah pada Inceptisols Effect of Silica Fertilizer on Lowland Rice Growth and Yield on Inceptisols. *Jurnal Tanah dan Iklim*, *42*(2), 153–160.

Suprihatno, B., Daradjat, A. A., Satoto, SE., B., Suprihanto, Setyono, A., Indrasari, S. D., Wardana, I. P., & Sembiring, H. (2010). *Deskripsi Varietas Padi 2018*. Balai Besar Penelitian Tanaman Padi.

Suthin Raj, T., Muthukumar, A., Muthukumaran, N., Sudhagar Rao, G., & Ann Suji, H. (2019). Induction of Defence Enzymes Activities in Rice Plant Treated by Seaweed Algae Against Rhizoctonia solani Kuhn Causing Sheath Blight of Rice. *Journal of Pharmacognosy and Phytochemistry*, *2*, 210–218.

Swain, R., & Rout, G. R. (2018). Effect Of Silicon Interaction With Nutrients In Rice. *Journal of Experimental Biology and Agricultural Sciences*, *6*(4), 717–731. https://doi.org/10.18006/2018.6(4).717.731

Taheri, P., & Tarighi, S. (2011). Cytomolecular aspects of rice sheath blight caused by Rhizoctonia solani. *European Journal of Plant Pathology*, *129*(4), 511–528. https://doi.org/10.1007/s10658-010-9725-7

Wang, M., Gao, L., Dong, S., Sun, Y., Shen, Q., & Guo, S. (2017). Role of silicon on plant–pathogen interactions. *Frontiers in Plant Science*, *8*(May), 1–14. https://doi.org/10.3389/fpls.2017.00701

Wang, Y., Wang, D., Shi, P., & Omasa, K. (2014). Estimating Rice Chlorophyll Content and Leaf Nitrogen Concentration with a Digital Still Color Camera Under Natural Light. *Plant Methods*, *10*(36), 1–11.

Yang, G., & Li, C. (2012). General Description of Rhizoctonia Species Complex. In *Plant Pathology*. https://doi.org/10.5772/39026

Yoshida, S. (1981). Fundamentals of Rice Crop Science. In *Fundamentals of rice crop science*.

Zhang, S. B., Zhao, L., Zhu, Z., & Zhang, Y. D. (2006). Genetic Analysis for Plant Height of a Semi-Dwarf Mutant 02428ha with Eui Gene in Rice. *Jiangsu Journal of Agronomic Science*, *22*(2), 100–104.

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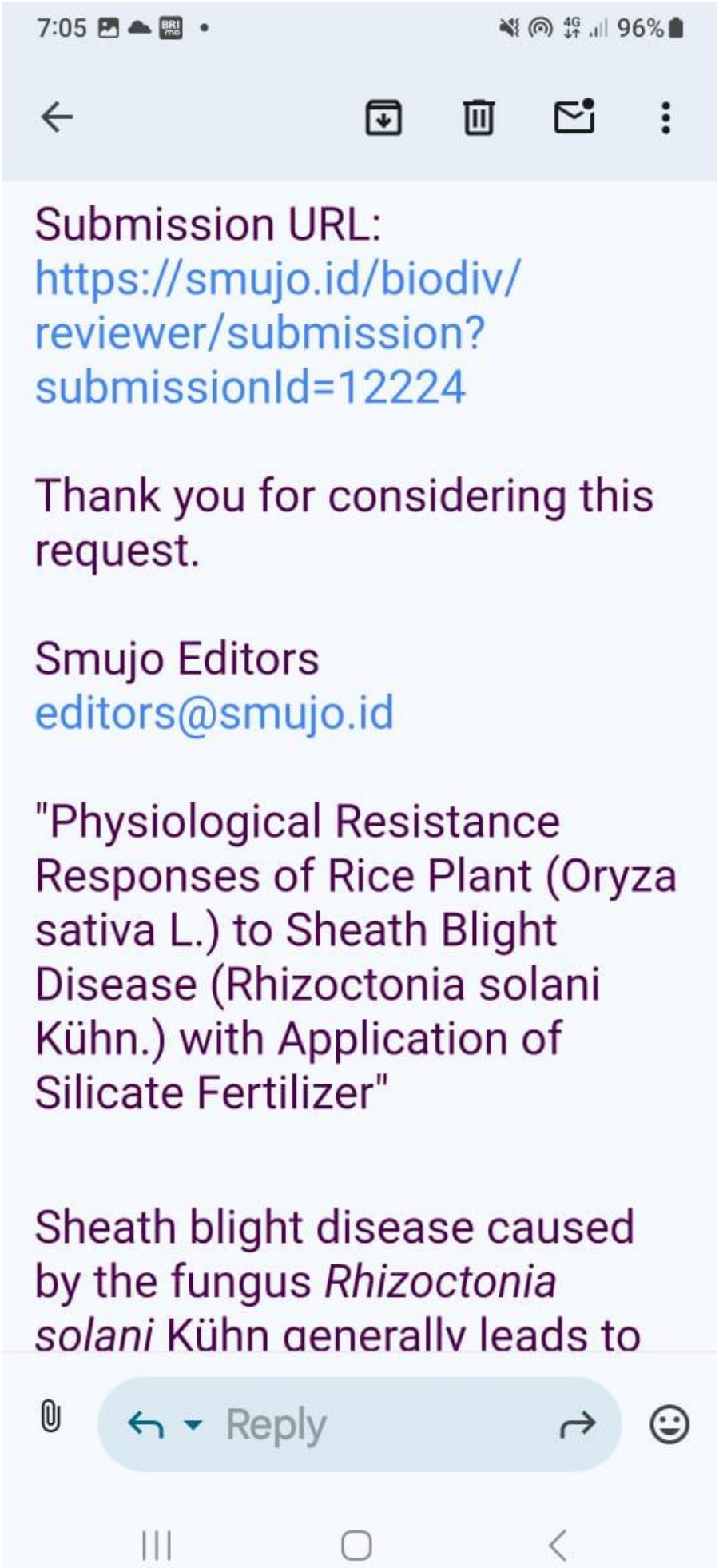
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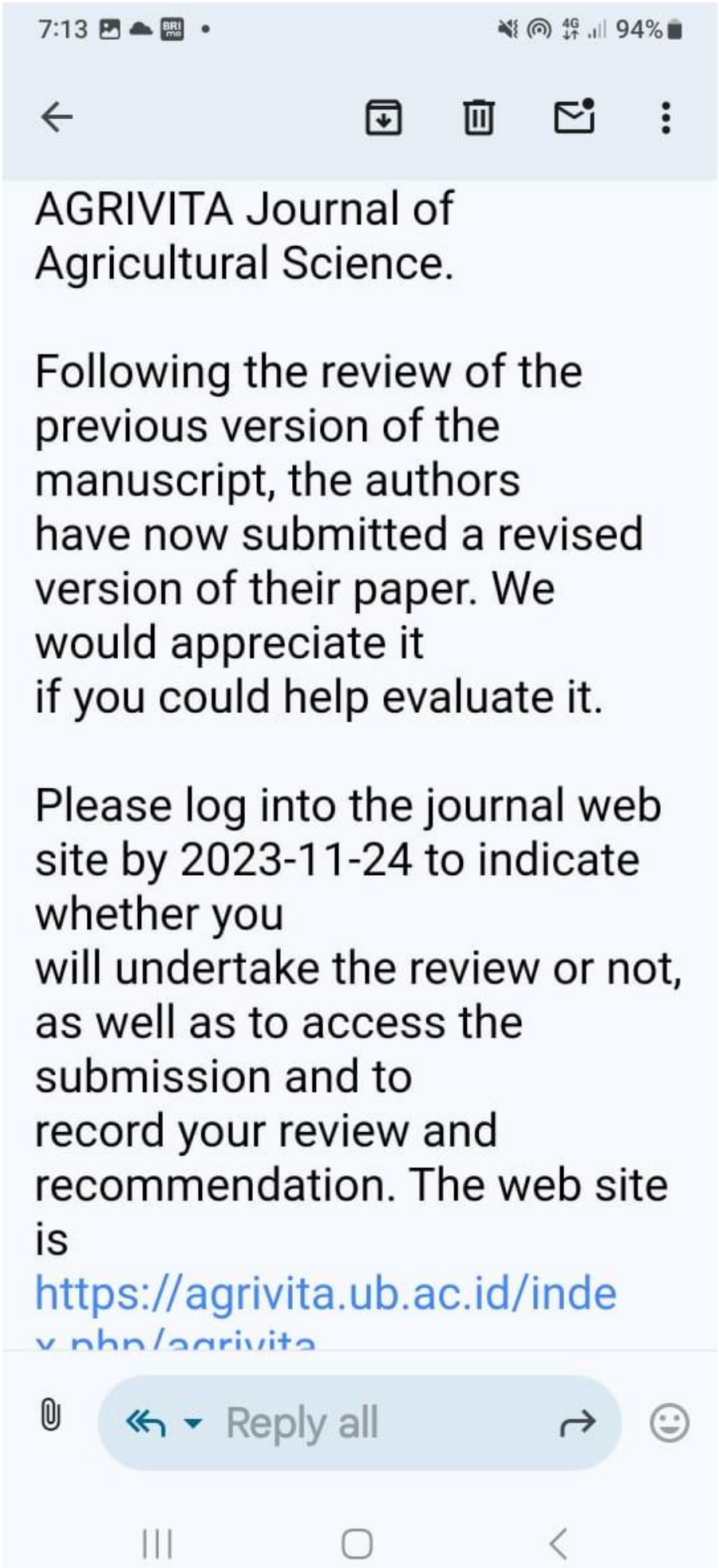
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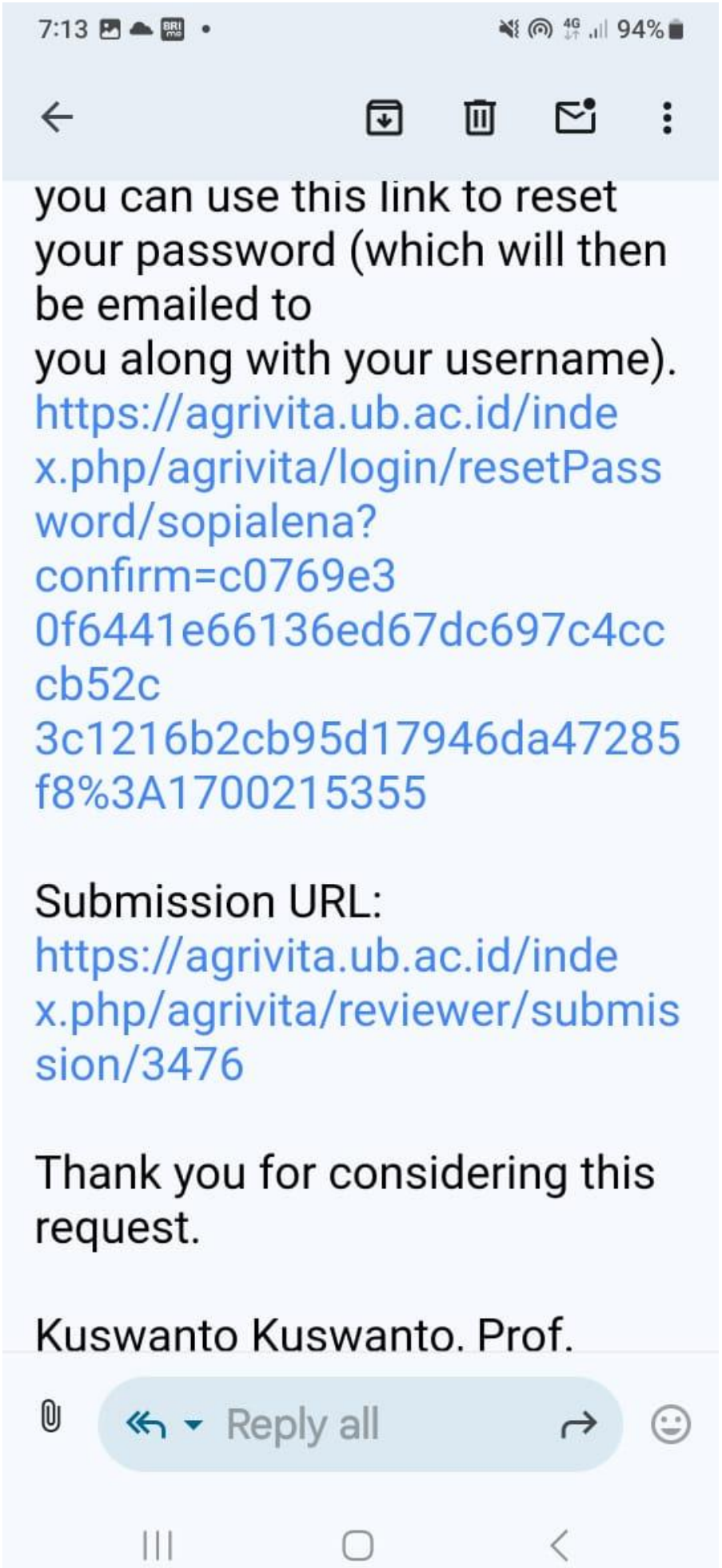
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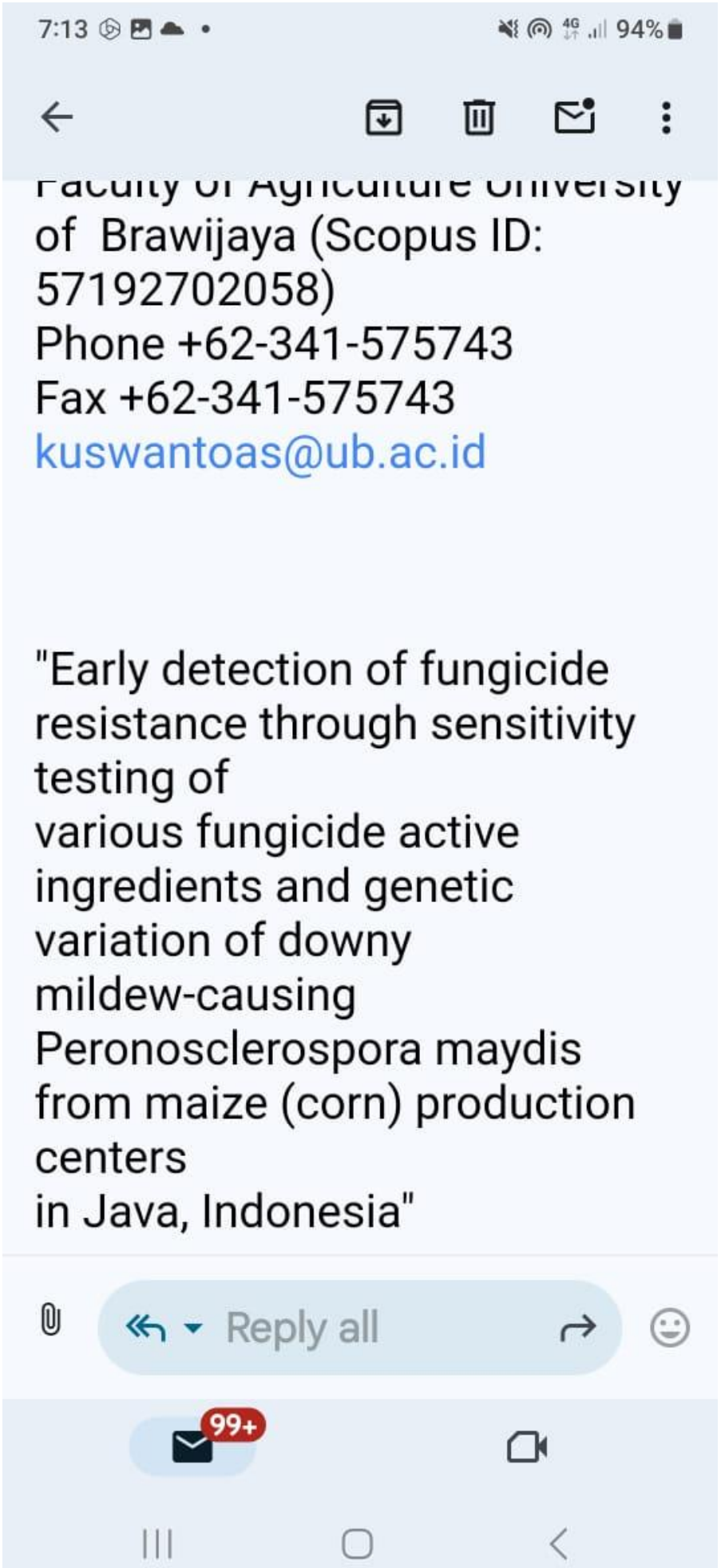
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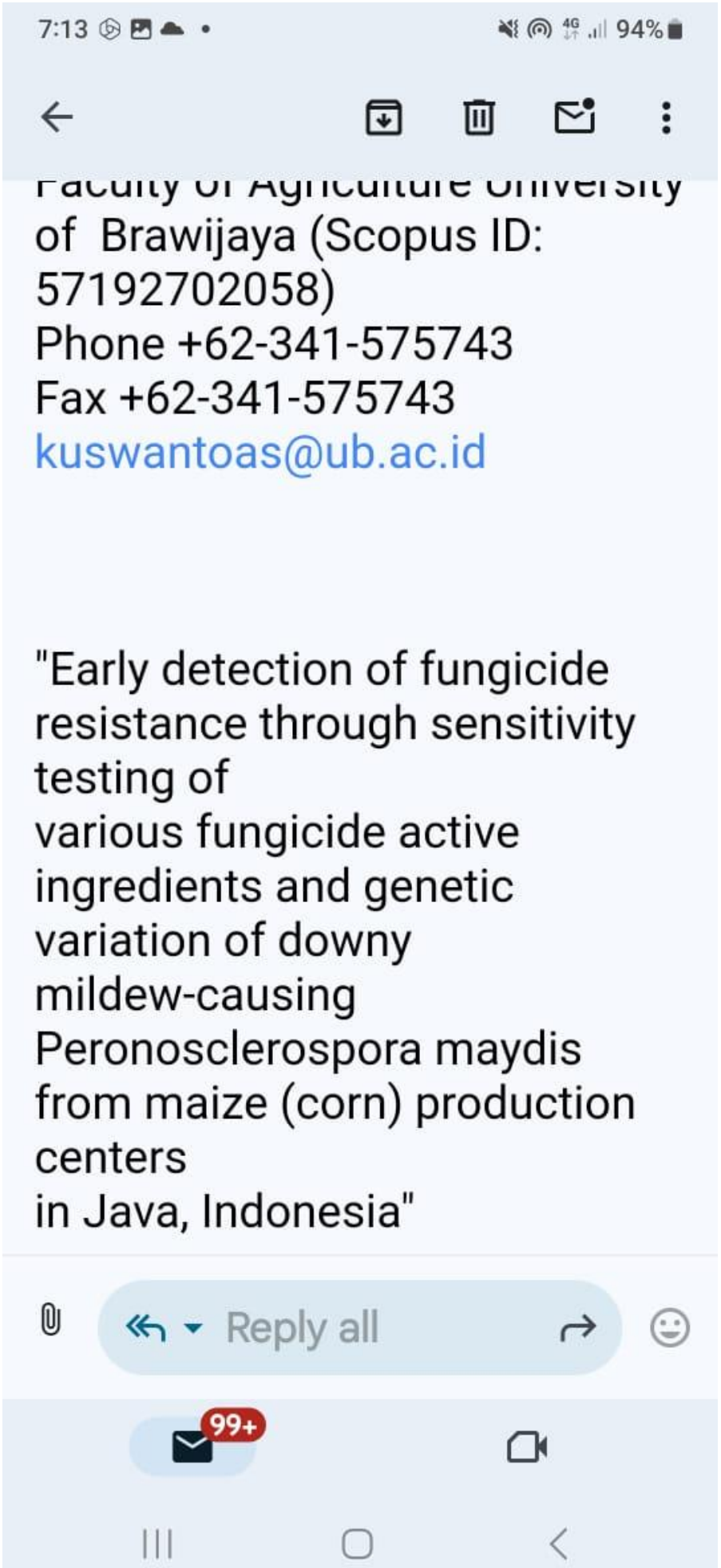
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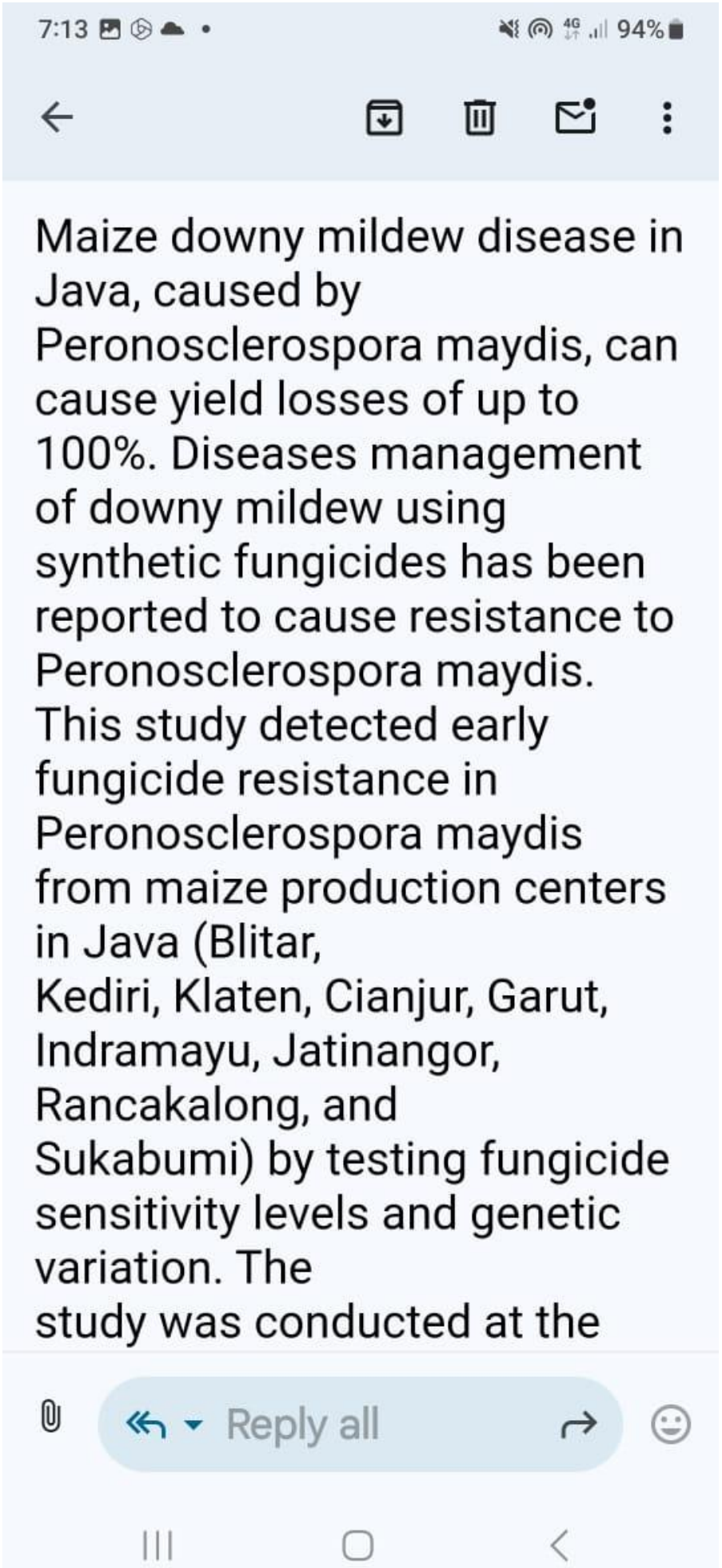
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Maize downy mildew disease in Java, caused by Peronosclerospora maydis, can cause yield losses of up to 100%. Diseases management of downy mildew using synthetic fungicides has been reported to cause resistance to Peronosclerospora maydis. This study detected early fungicide resistance in Peronosclerospora maydis from maize production centers in Java (Blitar, Kediri, Klaten, Cianjur, Garut, Indramayu, Jatinangor, Rancakalong, and Sukabumi) by testing fungicide sensitivity levels and genetic variation. The study was conducted at the
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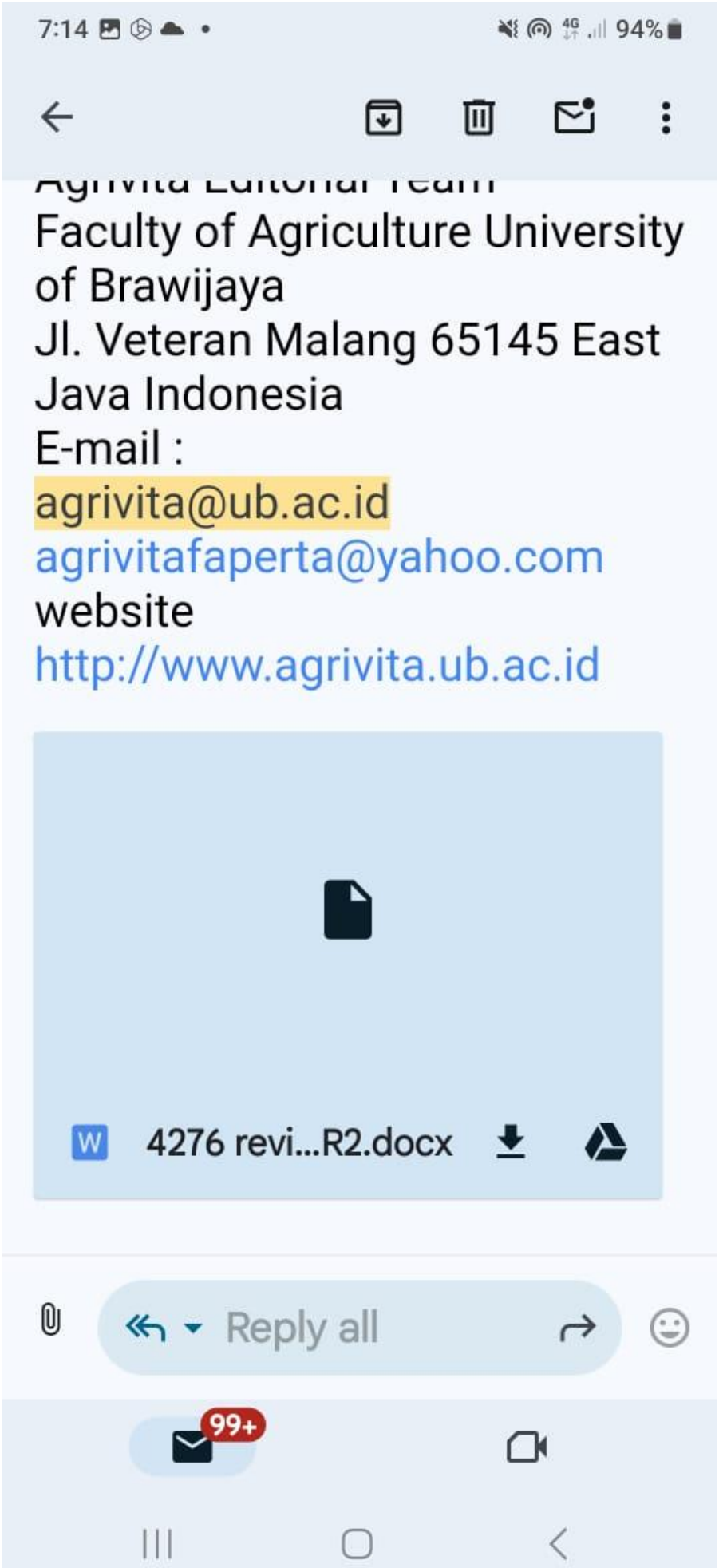
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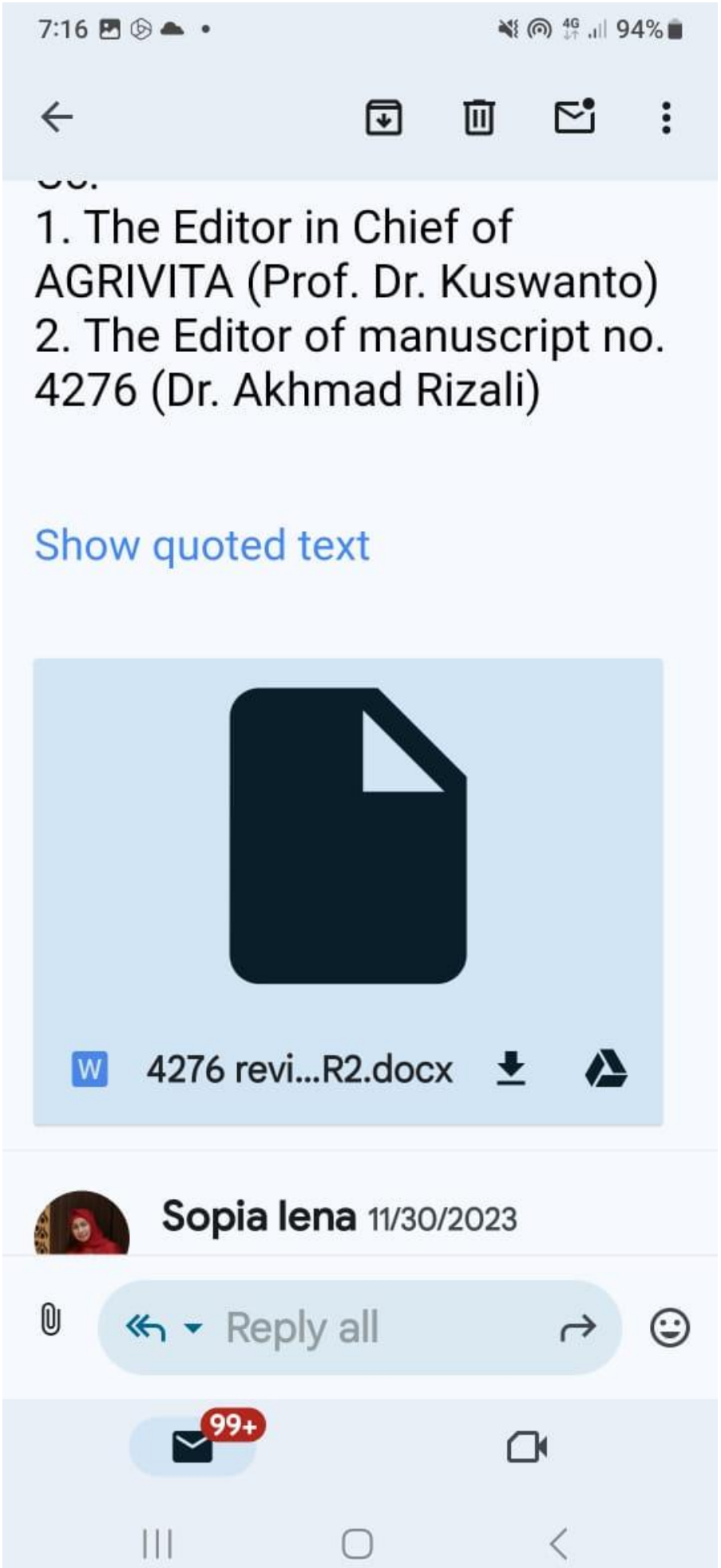
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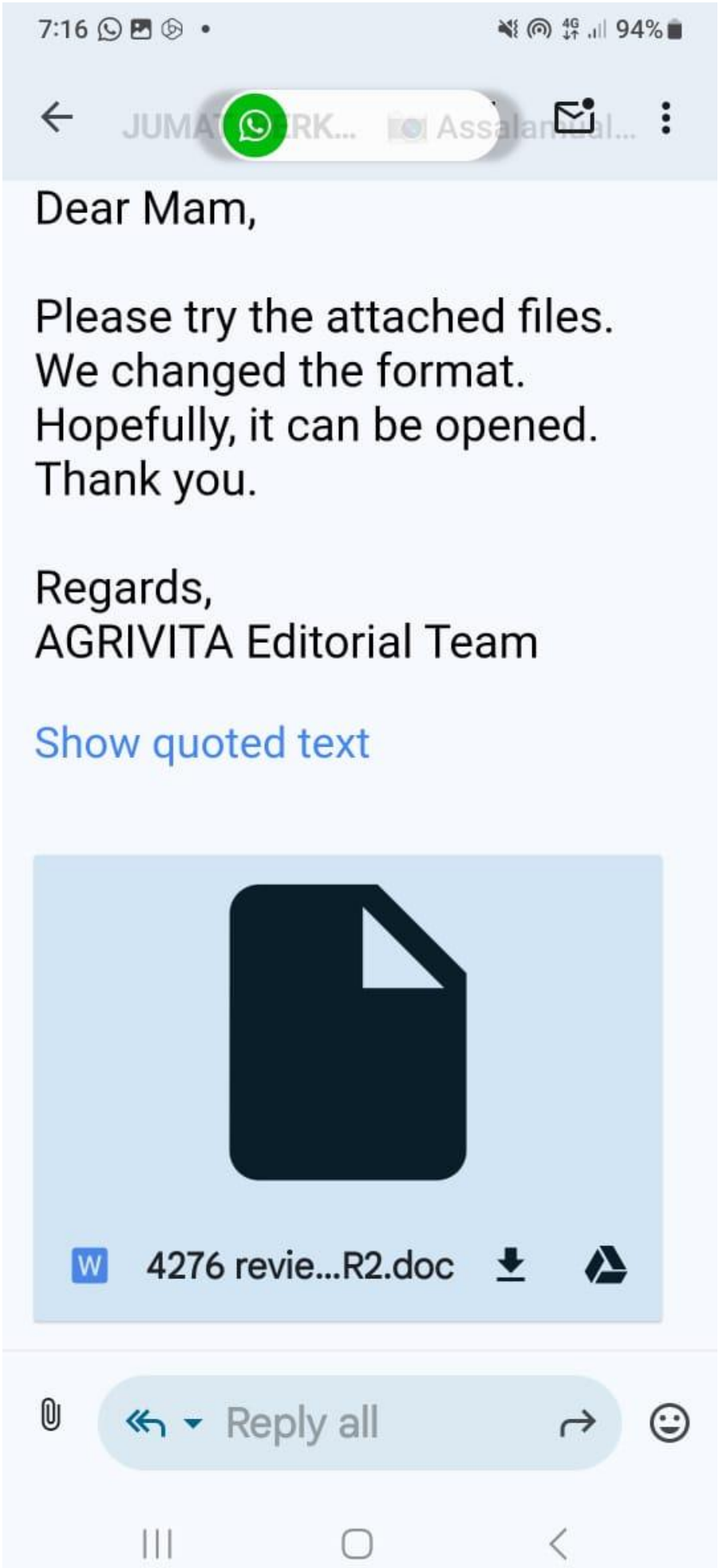
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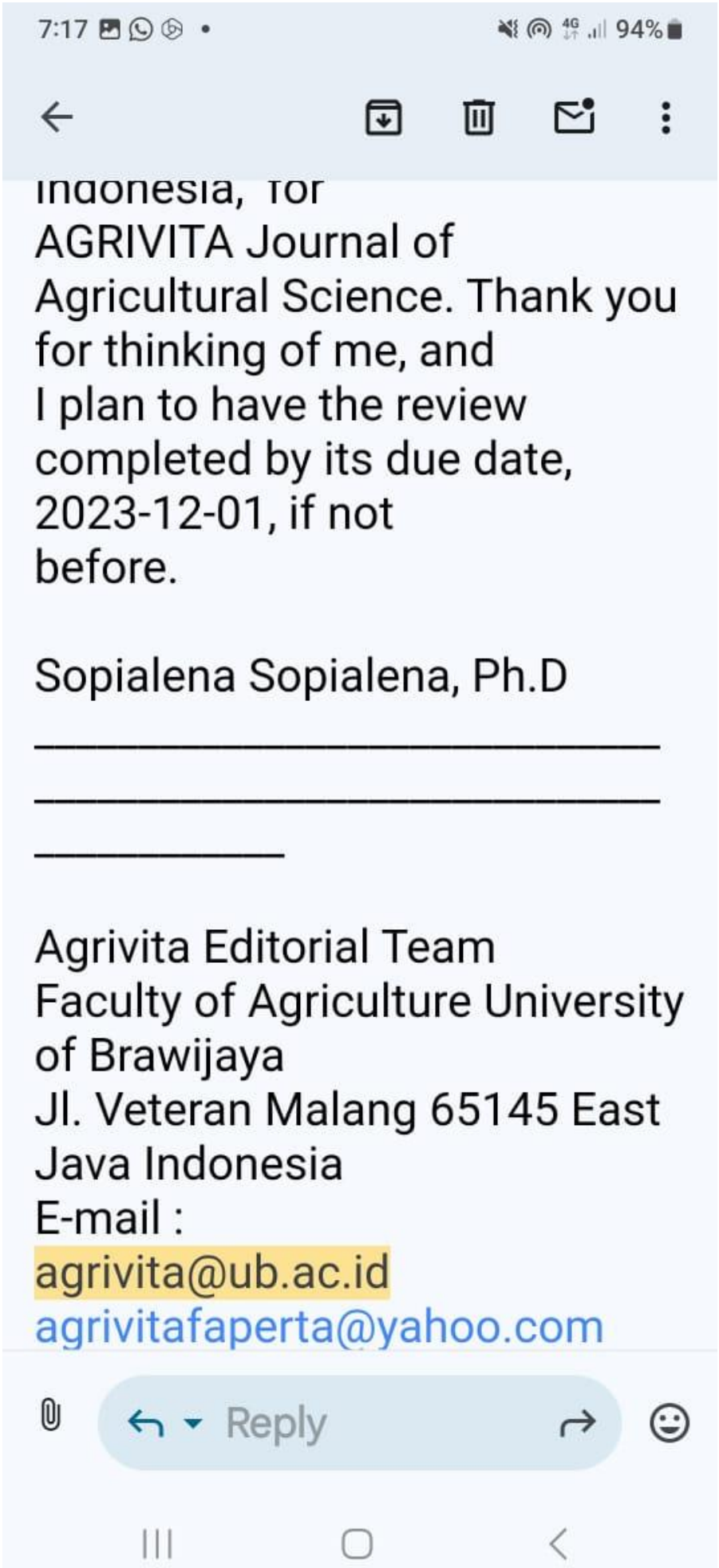
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I. Early detection of fungicide resistance through sensitivity testing of various fungicide active ingredients and genetic variation of downy mildew-causing *Peronosclerospora maydis* from maize (corn) production centers in Java, Indonesia

Satriyo Restu Adhi [1)], Fitri Widiantini [2*)] and Endah Yulia3 [)] (Arial 10)

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Early detection of fungicide resistance through sensitivity testing of various fungicide active ingredients and genetic variation of downy mildew-causing *Peronosclerospora maydis* from maize (corn) production centers in Java, Indonesia

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ABSTRACT

Maize downy mildew disease in Java, caused by *Peronosclerospora maydis*, can cause yield losses of up to 100%. Diseases management of downy mildew using synthetic fungicides has been reported to cause resistance to *Peronosclerospora maydis*. This study detected early fungicide resistance in *Peronosclerospora maydis* from maize production centers in Java (Blitar, Kediri, Klaten, Cianjur, Garut, Indramayu, Jatinangor, Rancakalong, and Sukabumi) by testing fungicide sensitivity levels and genetic variation. The study was conducted at the Laboratory of Biotechnology of Plant Protection Department of Plant Pests and Diseases, Faperta, Unpad. The results showed that all isolates generally still had sensitivity to dimetomorph, fenamidone, metalaxyl, and oxathiapiproline as evidenced by the presence of conidia damage although the amount varied. Isolates from Blitar, Kediri, and Klaten are indicated to be resistant to metalaxyl and fenamidone. While all isolates from West Java still show high sensitivity to metalaxyl. Oxathiapiproline was the most sensitive fungicide in damaging conidia of Peronosclerospora spp. in all locations. Based on the molecular test results there were intraspecies genetic variations that were seen in differences in the composition of nucleotides

KEYWORDS

Conidia, dimetomorph, fenamidone, metalaxyl, mutation, oxathiapiproline

INTRODUCTION

One of the important diseases in maize is downy mildew caused by *Peronosclerospora* spp (Crouch et al., 2022). The disease can cause a loss of world maize production by up to 30% (Rashid *et al* ., 2013) . The presence of downy mildew in Indonesia causes yield losses of 50-100% in susceptible plants (Lukman, 2012) . The high loss of maize yields is caused by downy mildew which can affect plant development at every growth stage (Rustiani *et al* ., 2015) .

Symptoms of maize downy mildew will generally be striped chlorosis on the leaves and the plants will become stunted (Leon, 2004) . Other symptoms will cause inhibition of vegetative and generative growth that can cause crop failure (Muis et al., 2018). Symptoms shown in the vegetative stage are the appearance of chlorosis in the leaf venation, the leaves will be shaped like a fan and the plant becomes stunted. Meanwhile, in the generative stage, downy mildew disease causes the cobs to not be covered and the seeds will not be fully filled (Rustiani *et al* ., 2015) . Signs of the presence of disease can be seen on the upper or lower surface of the leaves in the morning, that is, there will be a powdery white color which is the propagules of the conidia mass.

Downy mildew disease in maize in South-East Asia is caused by six different species, namely *Peronosclerospora maydis*, *Peronosclerospora philippinensis*, *Peronosclerospora sacchari*, *Peronosclerospora sorghi*, *Peronosclerospora neglecta,* and *Sclerospora rayssiae* var. *zeae* (Amran Muis et al., 2023; Rashid et al., 2013). Meanwhile in Indonesia, downy mildew is reported to be caused by four species, namely *P. maydis, P. philippinensis, P. sorghi,* and *P. neglecta* (Muis et al., 2023) . *P. maydis* infects maize plants in West Java, Central Java, Lampung, and Kalimantan; *P. sorghi* infects maize plants in the highlands of the Brastagi area, North Sumatra; *P. philippinensis* found on Sulawesi Island such as Gorontalo, North Sulawesi, and South Sulawesi; *P. neglecta* infects maize plants in several region in Sulawesi, East Java, and Kalimantan (Hikmawati et al., 2011; Muis et al., 2023; Widiantini et al., 2015).

One of the maize production centers is Java, which accounts for a total national contribution of 48.4% (Pusdatin, 2017) . The details of the contribution are divided into 27.7% from East Java Province; 15.0% from Central Java Province; and 5.7% from West Java Province (Pusdatin, 2017) . The spread of downy mildew in several maize production centers in Java has been reported. According to a report from the BBPOPT (2018) , East Java Province is an area with an area of infection downy mildew is the highest, followed by Central Java and West Java Provinces.

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Control efforts to minimize yield loss due to downy mildew can be carried out through technical culture by adjusting planting times, eradication, using resistant varieties, biological control, and chemical control (Frederiksen, 1980; Singh *et al*., 1987) . Chemical control using fungicides has been reported to cause many cases of resistance to plant pests. Possible risk emergence of resistance will be high if the fungicide is applied in frequent frequency in one growing season in the same area (Ishii, 2006) . According to Gisi & Sierotzki (2008) there are at least four active fungicidal ingredients that can be used to control downy mildew, namely phenylamides (eg metalaxyl or mefenoxam), carboxylic acid amides (eg dimetomorph), cyanoacetamidoximes (eg cyumoxanil), and quinone outside inhibitors (eg azoxystrobin , famoxadone, and fenamidone). Examples of fungicides from the phenylamide group include metalaxyl , which has been used frequently since 1983 (Hamilton, 2002; DEC, 2015) . Even in Indonesia itself, metalaxyl has become a control package recommended by the government in maize cultivation (Eliestya et al., 2014; Zubachtirodin et al., 2016) .

Pathogenic resistance to fungicides can occur because resistant strains of pathogens generally exist in nature due to natural mutations, but the application of fungicides can act as a selector for mutated strains (Bradley *et al.,* 2012) . According to Hobbleen *et al.* (2014) , the existence of pathogenic strains that are resistant to fungicides is passed through several stages, namely: (1) resistant strains are present due to natural mutations, the number is low and random , and (2) the application of fungicides is able to increase the number of resistant strains in the pathogen population because it acts as an agent s selectors. So that the existence of these stages will leave a population of pathogenic strains that are already resistant.

The method to determine the resistance status of pathogenic strains from certain regions can be done by testing their sensitivity to fungicides and looking at the various responses of conidial germination, morphological characteristics, then linked to their genetic profile (Janna, 2013) . Bock *et al.* (2000) reported that there were differences in morphological characteristics of *P. sorghi isolates* from several locations in Africa and showed different levels of pathogenicity. Morphological variations were also found in *P. maydis* isolates from various maize planting locations in Java, especially from the dimensions of the conidia and conidiophores (Widiantini *et al.,* 2015) . Differences in conidia and conidiophores sizes indicate genetic variation among *Pernonosclerospora maydis* isolates. Therefore, determining the presence of resistant strains from certain regions is an important step for an appropriate control management strategy.

MATERIALS AND METHODS

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Sampling of *P. maydis*

The research was conducted by purposively sampling isolates of Peronosclerospora maydis in East Java, Central Java, and West Java (Table 1) from November 2018 to August 2019. Then testing of fungicide sensitivity and genetic variation was carried out at the Plant Protection Biotechnology Laboratory, Faculty of Agriculture, Universitas Padjadjaran. The description of the sampling locations is in Table 1.

Table 1. Description of the sampling location.

Isolate Code	Location	Altitude (m asl)	Host	Fungicide	Disease incidence in the field (%)
BLT	Sukorejo Village, Sutojayan District, Blitar Regency -8.174 South Latitude; 112.247 East	169	Feed corn variety P18	Dimetomorph	15-20
KDR	Wonocatur Village, Ngasem District, Kediri Regency -7,774 South Latitude; 112.043 East	63	Feed corn variety P18	Dimetomorph	5-13
KLT	Bakung Village, Jogonalan District, Klaten Regency -7,737 South Latitude; 110.556 East	150	Feed maize varieties P27/P21	Metalaxyl	20-28

Isolate Code	Location	Altitude (m asl)	Host	Fungicide	Disease incidence in the field (%)
CJR	Sabandar Village, Karangtengah District, Cianjur Regency -6.815LS; 107.167 East	380	Talenta variety of sweet corn	Metalaxyl	10-15
IMY	Cikawung Village, Terisi District, Indramayu Regency -6,580LS; 108.036 East	49	Local corn variety (unknown)	Metalaxyl	4-10
JTN	Hegarmanah Village, Jatinangor District, Sumedang Regency -6,924 LS; 107.776 East	746	Secada sweet corn	Metalaxyl	40-45
GRT	Sadang Village, Sucinaraja District -7,189 South Latitude; 107.979 East	751	Talenta variety of sweet corn	Metalaxyl	30-35
RCG	Rancakalong Village, Rancakalong District, Sumedang Regency -6,854 LS; 107,840 East	865	Paragon sweet corn	Metalaxyl	30-40
SKB	Cimangkok Village, Suka Larang District, Sukabumi Regency -6,891LS; 107,001 East	814	Bisi 18 variety fodder maize	Metalaxyl	30-40

Fungicide Sensitivity Test on *P. maydis*

This experiment uses a linear model Completely Randomized Design Factorial pattern. The first factor used was the type of fungicides commonly used to control maize downy mildew in which containing different active ingredient which consisted of 4 levels, namely dimetomorph (Demorf™ 60 WP), fenamidone (Target™ 500 SC), metalaxyl (Saromyl™ 35 SD), and oxathiapiprolin (Plenaris™ 200 FS). The second factor was the dose/concentration of the fungicide, the level consisted of the recommended dose/concentration (X), 1/2X, 1/4X, and 1/8X for dimetomorph, fenamidone, metalaxyl, and oxathiapiprolin fungicides and 1 control level (aquadest). The third factor was isolates of Peronosclerospora maydis. origin of the sampling location in Java Island, which consists of 9 levels namely BLT, KDR, KLT, CJR, GRT, IMY, JTN, RCG, and SKB.

Each recommended dose/concentration for each fungicide active ingredient used in this test was dissolved in 10 ml of sterile distilled water. The test was carried out using a modified method from Widiantini *et al.* (2017) namely by mixing the conidia of *Peronosclerospora* isolates in each fungicide solution. Conidia were harvested from the leaves of corn infected with downy mildew using a brush slowly. Then 50 µl of fungicide + conidia suspension samples were transferred to a concave *glass object* and covered using *a cover glass*. Furthermore, the samples were incubated in a plastic box with a cover that had been covered with damp *tissue paper* for 24 hours in a dark room.

Observations were made by looking at the number of damaged conidia. Each treatment sample observed 50-55 conidia repeated 4 times, so that the total conidia observed were 200-220 conidia in each treatment. Damaged conidia are characterized by changes in conidial morphology such as lysis, incomplete shape, and damage to the cell wall. The number of damaged conidia is entered into the formula for the percentage of damaged conidia.

$$\text{Conidia damage (\%)} \frac{\text{Number conidia damaged}}{\text{Total number conida damaged}} \text{ x } 100\%$$

Analysis of statistical data on the percentage of conidia damage to fungicide in each area was analyzed by analysis of variance (ANOVA). The analysis program used is SPSS 21 and SASM-Agri. If the results of the F test on the fungicide and the area show that there is a significantly different interaction then proceed with the Scott-Knott follow-up test with a significance level of 5%.

Detection of *P. maydis Genetic Variation*

DNA extraction was carried out using the modified method of Mathiyazhagan *et al* . (2008) , starting with mixing 100 - 200 µl of conidial suspension harvested using a brush from downy mildew symptomatic plants in 500 µl of CTAB *buffer solution* (50 mM Tris-HCl, pH 8.0; 10 mM EDTA; 0.7 M NaCl; 1% cetyltrimethylammonium bromide (w/v); and 1% 2-mercaptoethanol) in a 2 ml Eppendorf tube. The solution was then vortexed for 30 seconds. Then incubated using *a dry bath* for 60 minutes at 60°C. after incubation the CTAB- *buffered conidial mixture* was centrifuged at 13,000 g for 10 minutes. Added 600 µl of chloroform:isoamylalcohol (CIA) solution and then incubated for 60 minutes on a *shaker machine* with a speed of 100 rpm and a temperature of 27 - 28ºC. After that it was centrifuged at 13,000 g for 10 minutes. Then the supernatant was transferred to a new Eppendorf tube and re-extracted again using chloroform:isoamylalcohol (CIA). Then the supernatant was transferred back to a new 1.5 ml Eppendorf tube and the DNA was precipitated by adding 600 µl of cold isopropanol, then centrifuged again at 13,000 g for 10 minutes. Then the DNA pellet was washed using 70% cold ethanol. After that the pellets were dried for 10 – 15 minutes and redissolved in TRIS-EDTA *buffer solution* (10 mM Tris-HCl and 1 mM EDTA, pH 8.0).

Quantification of the extracted DNA was carried out using the Nanodrop® Spectrophotometer ND-2000. The DNA template was taken 1 µl and read at a wavelength of 260 and 280 nm. The level of DNA purity is obtained from the absorbance ratio of 260/280 nm. Pure DNA is in the range 1.8 – 2.0. The quality test method for extracted DNA was carried out using an agarose gel electrophoresis method with a concentration of 0.8%. 0.8% agarose gel was prepared by dissolving 0.8 g in 100 ml of 0.5x TBE solution. Then it is heated using *a magnetic hot plate stirrer* until it boils. After that, 10 µl/100 ml of agarose was used for *staining.* The agarose gel is then poured into a mold and placed on a comb to make a well. A total of 2.5 µl of DNA sample was added with 0.5 µl *of loading dye* and put into the agarose well then electrophoresed at 70 Volts for 45 minutes (Mupid). DNA visualization was performed under *UV Transilluminator* (BluPAD).

DNA samples showing genomic DNA bands based on the results of the DNA quality test by electrophoresis were then subjected to the PCR process using a specific primer for *P. maydis* (Rustiani et al., 2015b) namely PmUF (5'- TCGTTATAGAAGCTAT(T/C)CATTAG -3') and PmUR (5'-GCCATCGAGTAATCCATTGTT -3'). A total of 5 µl DNA sample (concentration <100 ng/µl) was added with 12.5 µl KAPA2G *Fast ReadyMix PCR Kit+Dye*, 5 µl PCR *-grade water*, and primers PmUF and PmUR each 1.25 µl (what are the concentrations). The amplification program for the primer is an initial synthesis of 95° C for one minute, followed by 35 cycles which are divided into denaturation stages of 95°C for one-minute, annealing temperature of 57°C for one minute, extension temperature of 72° C for one 30 seconds, then the final synthesis is carried out at a temperature 72º C for five minutes. Amplification was carried out using a PCR machine (SelectCycler II Thermal Cycler).

The PCR product is then purified to clean and purify the DNA from impurities and reagents. Purification using *Geneaid Gel/PCR DNA Fragments Extraction Kit*. The procedure follows the manual instructions provided which begins by mixing the PCR product with DF *buffer* solution into a 1.5 ml Eppendorf tube with a volume of 1: 5. Then the PCR product and DF *buffer solution* are mixed using *a vortex* After mixing the suspension, put it in a pair of *DF column tubes* and 2 ml tubes. Then centrifuged at 16,000 g for 30 seconds.

The solution in the 2 ml tube was discarded and the 2 ml tube was reassembled with the *DF column tube*, then 600 µl *wash buffer was added* through the *DF column tube* and allowed to stand for one minute. Centrifuge at 16,000 g for 30 seconds. After that, the solution in the 2 ml tube was discarded, then reassembled with the *DF column tube*. The *DF column* tube was dried by centrifuging at 16,000 g for five minutes. After drying, the *DF column tube* was replaced with a new 1.5 ml Eppendorf tube. Then, 50 µl of *elution buffer* solution (Tris-HCL 10 mM, pH 8.5) was added to it via *the DF column* and allowed to stand for ten minutes. After that it was centrifuged at 16,000 g for ten minutes. The purified DNA is in a 1.5 ml Eppendorf tube.

Visualization of purified DNA was carried out using the agarose gel electrophoresis method. Agarose gel concentration of 1.7% and 100bp markers (Geneaid) were used at 70 Volts for 45 minutes (Mupid). Furthermore, DNA visualization was carried out under *UV Transilluminator* (BluPAD). The purified DNA obtained was analyzed *for sequencing* at Macrogen Korea using the ABI PRISM 377 DNA Sequencher to obtain the nucleotide sequences.

Analysis of genetic variation begins with carrying out *DNA barcoding procedures*. The nucleotide sequences obtained from the *sequencing results* were subjected to homology analysis on the *Basic Local Alignment Search Tool Nucleotide* (BLASTN) – *National Center of Biological Information* (NCBI) website (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Homology analysis aims to match the data available in the NCBI and *GenBank databases.*

The genetic diversity of *P. maydis isolates* was identified by the nucleotide sequence alignment comparison method between isolates and accessions from *Genbank*. The alignment of the nucleotide sequences of *P. maydis isolates* from several locations in Java Island was initially edited/ *contiguous* using the ClustalW method in the BioEdit software. In addition, genetic diversity and kinship between isolates were further analyzed using MEGA-X software through the *Neighbor Joining* (NJ) method with a *bootstrap value of* 1000x repetitions in the phylogenetic dendrogram construction. Analysis of diversity based on amino acids was carried out by translating nucleotide sequences into amino acids using the MEGA-X software. Variations, mutation points, and areas of conservation of amino acids from each isolate were viewed using the Jalview software.

RESULTS AND DISCUSSION

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Sensitivity of *P. maydis* to 4 Fungicide Concentration Levels

Treatment of the four fungicide active ingredients at four concentration levels produced a significant effect on conidia damage of *Peronosclerospora maydis* tested. This influence shows varying values. This is listed in Table 2.

Table 2. Damage to conidia (%) of *Peronosclerospora* spp. from several locations in Java Island which were treated with dimetomorphic fungicides, fenamidone, metalaxyl, and oxathiapiprolin.

Fung	BLT	KDR	TLC	CJR	GRT	IMY	JTN	RCG	SKB
K	0 a A	0 a A	0 a A	0 a A	0 a A	0 a A	0 a A	0 a A	0 a A
DX	11.5 a E	18.9 b G	28.9 c G	19.5 b D	28.2 c D	39 d D	39 d E	26 c B	21.5 b B
D1/2	7 a D	15.8 b F	20.5 b E	17.5 b C	21.2 b C	31.5 d C	35 d D	26 c B	19.5 b B
D1/4	4 a C	11.5 b E	15 b D	16 b C	20.5 c C	30.5 d C	24 c B	26 d B	19 c B
D1/8	2.5 a B	10,1 b E	11.8 b D	14 b C	14,3 b B	28.5 d C	25,5 d B	22 c B	18.5 c B
FX	5.9 a D	7.4 a D	23.2 b F	28 c E	31 c D	38.5 d D	43.5 d E	29.5 c C	33 c C
F1/2	2.5 a B	5,9 a C	16,4 b D	20 b D	19.8 b C	38 d D	38.5 d E	28 c B	30.5 c C
F1/4	1 a A	5,5 a C	13,9 b D	14 b C	18.5 b C	33 d C	35,5 d D	26 c B	29.5 c C
F1/8	2 a	4.5 a	9.7 b	8.5 b	13.6 c	30.5 f	31.5 f	26 e	19 d

Fung	BLT	KDR	TLC	CJR	GRT	IMY	JTN	RCG	SKB
	B	C	C	B	B	C	C	B	B
MX	5,4 a	9.1 a	18.4 b	51.5 d	22,3 b	30 c	30 c	31 c	44.5 d
	D	E	E	G	C	C	C	C	D
M1/2	4 a	5 a	14 b	33 e	16,2 b	26.5 c	29.5 d	30 d	33.5 e
	C	C	D	F	D	C	C	C	C
M1/4	2 a	3.9 a	13.9 b	22 c	15,3 b	18.5 c	29.5 d	26.5 d	32.5 d
	B	C	D	D	B	B	C	B	C
M1/8	0.5 a	2,5 a	6.5 b	13 c	14.4 c	17.5 d	23 d	23.5 d	28 f
	A	B	B	C	B	B	B	B	C
OX	14,2 a	20,7 b	47.8 d	34 c	37.3 c	46.5 d	44 d	47.5 d	31 c
	F	G	J	F	E	E	E	D	C
O1/2	7 a	17.1 b	35.3 e	28.5 d	34.5 e	41 f	39 f	35 e	24.5 c
	D	F	I	E	E	D	E	C	B
O1/4	4,5 a	9,6 a	31.6 d	27.5 c	33.5 d	19.5 b	36 d	34 d	23.5 c
	C	E	H	E	E	B	D	C	B
O1/8	3,5 a	7.5 a	28 d	22 c	22.2 c	14 b	22 c	24.5 c	21 c
	C	D	G	D	C	B	B	B	B

Remarks: The same letter in one line shows results that are not significantly different based on the Scott-Knott test at the 5% level; The same letter in one column shows results that are not significantly different based on the Scott-Knott test at the 5% level; D (Dimetomorph), F (Fenamidone), M (Metalaxyl), O (Oxathiapiprolin), K (Aquadest).

The use of metalaxyl at the recommended concentration (packaging label) produced a different effect on each *Peronosclerospora* spp. Referring to the percentage value of damaged conidia (Table 2, column MX) metalaxyl is generally still effectively used in West Java, but not in Central Java and East Java. The metalaxyl treatment caused a significantly higher percentage of conidia damage in some *Peronosclerospora spp.* isolates taken from areas in West Java were compared with BLT, KDR , KLT , and GRT isolates. The percentage of damaged conidia in Blitar, Kediri, Klaten, and Garut showed the lowest values. In contrast to the five isolates from West Java which still showed sensitivity to metalaxyl, although at concentrations lower than recommended.

Furthermore, damage to the conidia of *Peronoscleropsora* spp. generally tend to be found to be lower in isolates from Blitar, Kediri and Klaten when compared to isolates from other areas. Not only for metalaxyl, but for other fungicide active ingredients, except for oxathiapiprolin. This shows that the isolates from Blitar, Kediri and Klaten are indicated to be resistant to fungicides with certain active ingredients. For example, isolates from Blitar, Kediri and Klaten were suspected of showing resistance to the fungicide fenamidone. Indications of fenamidone resistance can be seen in the percentage value of conidia damage from Blitar and Kediri isolates (Table 8, column FX) which is low and significantly different when compared to isolates from other regions.

Kediri and Blitar are corn-growing centers in East Java. The harvested area in 2017 was around 51.273 ha for Kediri and 52,098 ha for Blitar (Badan Pusat Statistik, 2018b) . Meanwhile, Klaten in 2017 had a harvested area of 11.085 ha (Badan Pusat Statistik, 2018a) . Intensive maize cultivation for several years in the area is thought to be one of the causes of metalaxyl and fenamidone fungicides becoming resistant to downy mildew. Intensive crop cultivation will certainly increase agricultural external inputs such as inorganic fertilizers, pesticides, monoculture cultivation, and mechanization (Kughur & Audu, 2015) . This will have an impact on several things such as the environment, biodiversity, and human life. The relationship between intensive cultivation and plant disease will continue, causing an increase in the number of inoculums and the intensity of attacks (Waceke & Kimenju, 2007) .

Comment [A18]: used maize instead of corn

The decrease in metalaxyl and fenamidone sensitivity is thought to be due to mutations in the species *Peronosclerospora maydis* from Kediri and Blitar. Naturally in nature there are sensitive pathogenic strains and pathogens resistant to fungicides. Continuous fungicide application will reduce the number of sensitive strains and increase the number of resistant pathogenic strains. In addition, sensitive pathogenic strains are also capable of changing properties due to accumulation of mutated genes (Damicone, 2008) . According to Sierotzki *et al.* (2019) Phenylamide fungicides such as metalaxyl cause single gene mutations (monogenic) in target organisms. Metalaxyl has a mechanism of action inhibiting the activity of nucleic acid biosynthesis in RNA polymerase 1 and exerts a concurrent effect on mitosis (Fisher & Hayes, 1984, 1982; Yang *et al* ., 2011) .

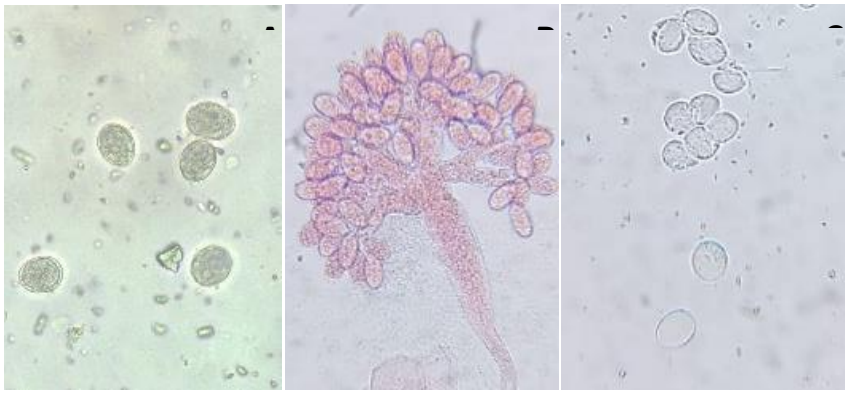

Figure 1. Conidia isolates *Peronosclerospora* spp. (A) Normal conidia, (B) Normal conidia in metalaxyl treatment, (C) Damaged conidia

Randall *et al* . (2014) stated that an amino acid (AA) mutation in the RNApoll gene (RPA190) which encodes the RNA polymerase I subunit in oomycete *P. infestans* will be associated with a decrease in the sensitivity of metalaxyl and mefenoxam fungicides. According to Chen *et al* . (2018) generally found two *pathways* for changes in the mechanism of metalaxyl resistance in *P. infestans.* The first *pathway is that the RPA190 gene in a metalaxyl sensitive isolate undergoes a single amino acid mutation that changes from vialine to glycine at the amino acid position 1476 (V1476G).* While the second *pathway* will involve mutations at several amino acid positions such as R296H, F382Y, P980S, E1174A, D1228E, V1476G, D1546Y, and P1600S. Changes in amino acids are thought to have an effect on metabolic processes in target organisms.

A decrease in the sensitivity of QoI (*quinone outside inhibitors*) fungicides such as fenamidone has been reported. According to Gisi & Sierotzki (2015) QoI fungicides, for example the active ingredient fenamidone, have a high risk of causing oomycete resistance. Reported by Toffolatti *et al*. (2011) that QoI fungicide has caused resistance in *P. viticola* due to mutations of the G143A amino acid in the mitochondrial cytochrome b gene region. Fenamidone is a QoI fungicide which has a mechanism of action by inhibiting mitochondrial respiration by inhibiting electron transfer in cytochrome b (complex III) (Gisi & Sierotzki, 2008) .

The fungicide with the active ingredient oxathiapiprolin at four concentration levels (X, 1/2X, 1/4X, and 1/8X) in this experiment resulted in the highest percentage of conidia damage in almost all sampling locations (Table 8). This is presumably because oxathiapiprolin is an active ingredient that was only sold to the market in 2005 and is an active ingredient specifically made to control oomycetes (oomisides) (Cohen, 2015) . The mechanism of action of oxathiapiprolin is by inhibiting homologous *oxysterol binding protein* (OSBP) which plays a role in the movement of lipid compounds between membranes which interferes with signaling processes, maintains cell membranes, and forms complex lipid compounds which are important for oomycete cells to stay alive (Pasteris et *al* . , 2015; FRAC, 2017, 2018) .

The active ingredients of dimetomorph and fenamidone fungicides also have different sensitivity values. Isolate *Peronosclerospora* spp. from Klaten, Kediri, and Blitar respectively have a tendency to be sensitive to dimetomorphs then phenamidon. While some isolates from West Java have the opposite tendency. This difference is thought to be due to the influence of the range, frequency, and rotation of the fungicide in the area which affects the response of the target organism.

Identification of *P. maydis*

Use of specific primers for *P. maydis* ie PmUF and PmUR succeeded in amplifying the DNA of *Peronosclerospora* spp. extraction results from eight areas in Java Island. Based on visualization (Figure 2) all *Peronosclerospora* spp isolates were confirmed as *P. maydis.* The PmUF and PmUR primers have a target size of about 304 bp.

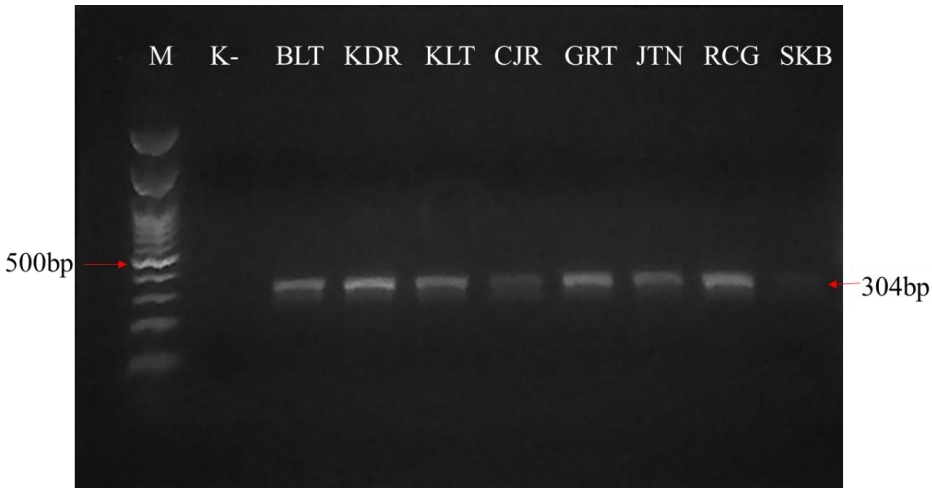

Figure 2. Visualization of DNA fragments using *P. maydis* specific primers PmUF and PmUR on 1.7% agarose gel. (M) 100bp DNA marker (Geneaid), (K-) Negative control (aquades), (BLT) P. maydis isolate from Blitar, (KDR) Kediri isolate, (KLT) Klaten isolate, (CJR) Cianjur isolate , (GRT) Garut isolate, (JTN) Jatinangor isolate, (RCG) Rancakalong isolate, and (SKB) Sukabumi isolate.

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Based on the results of molecular identification and homology analysis (Table 2), all isolates were identified as *P. maydis*. Isolates from Blitar, Kediri, Klaten, Cianjur, Garut, Jatinangor and Sukabumi have similarities with accession HM988978.1 from Malang which has been deposited at *GenBank.* Meanwhile, the isolate from Rancakalong had similarities with the accession HM988976.1 from Malang. This shows that the distribution of *P. maydis species* from all sampling locations is related. According to Bonde (1982) , *P. maydis* was first reported as a cause of downy mildew in Java since 1897 by Raciborski.

Table 1. Identification results, similarity (%), specimen/origin, and *Genbank* accession numbers with nucleotide sequences available in the NCBI database.

Isolate	Identification Results	Similarity (%)	Specimen/ Origin	*GenBank* Accession Number
BLT	*Peronosclerospora maydis* isolate 123062 cytochrome oxidase subunit II (COXII) gene , partial cds; COXII-COXI intergenic spacer, complete sequence; and cytochrome oxidase subunit I (COXI) gene, partial cds; mitochondrial	97.86	*P. maydis* Malang	HM988978.1
KDR	*Peronosclerospora maydis* isolate 123062 cytochrome oxidase subunit II (COXII) gene , partial cds; COXII-COXI intergenic spacers, complete sequence; and cytochrome oxidase subunit I (COXI) gene, partial cds; mitochondrial	97.50	*P. maydis* Malang	HM988978.1
KLT	*Peronosclerospora maydis* isolate 123062 cytochrome oxidase subunit II (COXII) gene , partial cds; COXII-COXI intergenic spacers, complete sequence; and cytochrome oxidase subunit I (COXI) gene, partial cds; mitochondrial	96.43	*P. maydis* Malang	HM988978.1
CJR	*Peronosclerospora maydis* isolate 123062 cytochrome oxidase subunit II (COXII) gene , partial cds; COXII-COXI intergenic spacers, complete sequence; and cytochrome oxidase subunit I (COXI) gene, partial cds; mitochondrial	98.21	*P. maydis* Malang	HM988978.1
GRT	*Peronosclerospora maydis* isolate 123062 cytochrome oxidase subunit II (COXII) gene , partial cds; COXII-COXI intergenic spacers, complete sequence; and cytochrome oxidase subunit I (COXI) gene, partial cds; mitochondrial	98.89	*P. maydis* Malang	HM988978.1
JTN	*Peronosclerospora maydis* isolate 123062 cytochrome oxidase subunit II (COXII) gene , partial cds; COXII-COXI intergenic spacer, complete sequence; and	96.79	*P. maydis* Malang	HM988978.1

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Isolate	Identification Results	Similarity (%)	Specimen/ Origin	*GenBank* Accession Number
	cytochrome oxidase subunit I (COXI) gene, partial cds; mitochondrial			
RCG	*Peronosclerospora maydis* isolate 310106 cytochrome oxidase subunit II (COXII) gene , partial cds; COXII-COXI intergenic spacer, complete sequence; and cytochrome oxidase subunit I (COXI) gene, partial cds; mitochondrial	97.19	*P. maydis* Malang	HM988976.1
SKB	*Peronosclerospora maydis* isolate 123062 cytochrome oxidase subunit II (COXII) gene , partial cds; COXII-COXI intergenic spacer, complete sequence; and cytochrome oxidase subunit I (COXI) gene, partial cds; mitochondrial	98.60	*P. maydis* Malang	HM988978.1

All isolates that were identified had similarities to the species *Peronosclerospora maydis* isolates 123062 and 310106 cytochrome oxidase subunit II (COXII) gene, partial cds; COXII-COXI intergenic spacers, complete sequence; and cytochrome oxidase subunit I (COXI) gene, partial cds; mitochondrial. The use of the COX region to identify the oomycete class has been reported. Robideau *et al*. (2011) reported that the *DNA barcoding method* in the cytochrome oxidase subunit I (COXI) gene region is a new step to identify the oomycete group. In addition, Hudspeth *et al* . (2000) also reported that the COXII region can be used to identify the genetic relationships of 15 *Peronosporomycete species*. According to Kammarnjesadakul *et al*. (2011) the use of the COXII gene region was able to separate species to the intraspecific level.

The use of the COX gene region can also play a role in identifying species and their kinship in a location. Studholme *et al*. (2019) conducted a phylogenetic analysis of *P. kerinoviae* that causes stem canker disease in COXI-based forest plants from Chile, which turned out to have a close relationship with *P. kerinoviae* from New Zealand. Rustiani (2015) also reported that *P. sorghi* from NTT is similar to *P. sorghi* from Texas. Analysis based on a combination of COX and ITS was also able to find a new species of *P. subutonaiense* from China which is closely related to *P. utonaiense* from Japan (Chen & Bo, 2019) . Identification of this species kinship is also useful in supporting quarantine activities in a location. According to Gao & Zhang (2013) *DNA*- based identification *barcoding* will play an important role in ensuring the security of a location from the Quarantined Plant Pest Organisms (OPTK). Therefore, the spread of species that may have never been found and are more pathogenic can be prevented.

Genetic Variation *of P. maydis*

Neighbor Joining (NJ) method with a *bootstrap value* of 1000x repetitions resulted in a dendrogram that was divided into five clusters (Figure 3). The first cluster consists of isolates from Blitar, Garut and Klaten. The second cluster consists of isolates from Rancakalong, Kediri and Jatinangor. The third cluster consists of isolates from Cianjur. Sukabumi Isolate is in cluster four. Meanwhile, *GenBank* accessions HM988976.1 and HM988978.1 from Malang formed their own group in cluster five.

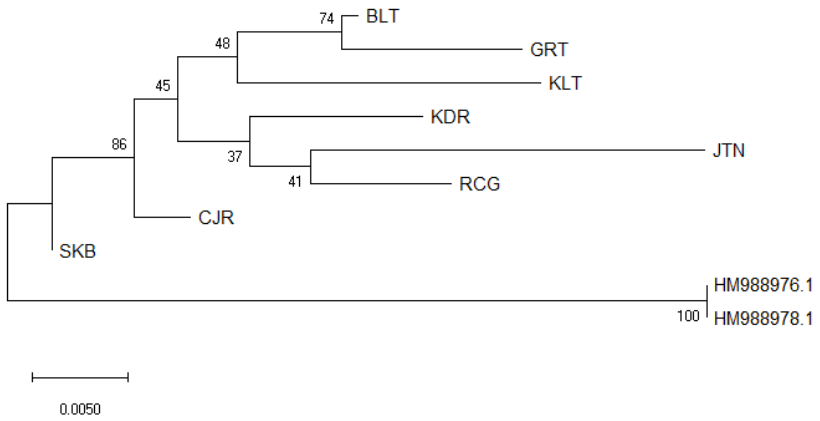


Figure 3. Relationship dendrogram of *P. maydis isolates* from several locations on Java Island and *GenBank* (HM) accession. The dendrogram is generated from the Neighbor Joining (NJ) method with a bootstrap value of 1000x repetitions

P. maydis isolate from Java Island, even though it is in the same species, has differences at the intraspecific level (Figure 3). The combination of isolates in one cluster is suspected due to the similarity factor based on the characteristics of the sampling location (Table 1). For example, in the second cluster, the sampling locations, namely in Rancakalong, Kediri, and Jatinangor, based on the author's observations during the research and interviews with local farmers (unpublished) are endemic areas of downy mildew where disease incidence occurs every season, although at a low percentage.

Muis *et al.* (2016) reported that seven isolates identified as *P. maydis* from Kediri were grouped into the same cluster based on closest genetic kinship. Kediri Regency is one of the areas where downy mildew is endemic (Burhanuddin, 2015) . Adhi et al. (2022) also reported that *Peronosclerospora* spp. in Java Island has diverse variations based on the morphology and morphometry of each isolate. Another example of Thai strain *P. insidiosum* taken from relatively the same source, namely water, has a tendency to join in the same cluster after *Neighbor Joining* (NJ) analysis (Kammarnjesadakul *et al* ., 2011) . Thus the kinship of each isolate that is in the same cluster is thought to be influenced by the similarity of the characteristics of the sampling location or the environment.

According to Lukman *et al*. (2012) variations among *P. maydis species* from several regions in Java Island are suspected to be due to high genetic variation or the presence of other species that infect maize plantations. This is supported by the statement of Muis *et al*. (2016) which states that if there is a reasonableness in an area there is more than one type of *Peronosclerospora* spp. due to environmental factors such as wind, water, soil, and circulation of seeds from another area, it supports the distribution of *Peronosclerospora* spp.

The limitation of this study is the small number of maize production centers sampled, namely 13 maize centers on the island of Java, so additional samples are needed in order to see wider variations in resistance and genetics. However, from the existing literature, the detection of resistance and genetics of *P. maydis* in the locations that have been studied along the island of Java has not been reported, a challenge that we will solve in the next study.

CONCLUSIONS AND SUGGESTIONS (Arial 10)

Peornosclerospora maydis have different sensitivities according to their location to the four active ingredients of the fungicide. There have been indications of resistance to metalaxyl and fenamidone in East and Central Java, but not in part in West Java. All *P. maydis* isolates to oxathiapiprolin even in 1/8 recommended concentration. Isolates from East Java and Central Java tend to be more sensitive to dimetomorph than those from West Java. While isolates in West Java tend to be more sensitive to fenamidone than those in East Java. There are intraspecific variations in P. maydis which can be proven by the formation of clusters based on phylogenetic analysis.

REFERENCES

Comment [A21]: Please complete the highlighted references with specific link.

The author should use reference tools such as Mendeley. All references mentioned should be written down in reference using the American Psychological Association (APA) style and arranged from A to Z. Articles have 10 years recent references and 80% is journal. The most of references are primary ones (last five years). **Unpublished data and personal communication should not be cited as literature citations.** "In Press" articles that have been accepted for publication may be cited in references. Include in the citation of the journal in which the "in press" article will appear and the publication date, if a date is available. **References consist of a minimum of 20 references.**

Adhi, S. R., Widiantini, F., & Yulia, E. (2022). Variasi Morfometri dan Patogenisitas *Peronosclerospora* spp. Penyebab Penyakit Bulai Jagung di Pulau Jawa, Indonesia. *Jurnal Fitopatologi Indonesia*, *17*(5), 173–182. https://doi.org/10.14692/jfi.17.5.173-182

Badan Pusat Statistik. (2018a). *Provinsi Jawa Tengah Dalam Angka 2018*. Badan Pusat Statistik Provinsi Jawa Tengah. https://jateng.bps.go.id/publication/2018/08/16/73e4da7b5902cb91ff591bc7/provinsijawa-tengah-dalam-angka-2018.html

Badan Pusat Statistik. (2018b). *Provinsi Jawa Timur Dalam Angka 2018*. Badan Pusat Statistik Provinsi Jawa Timur. https://jatim.bps.go.id/publication/2018/08/16/9999b727d316c006ee2fd7e7/provinsi-jawa-timur-dalam-angka-2018.html

BBPOPT. (2018). *Evaluasi dan Prakiraan Serangan Organisme Pengganggu Tumbuhan Utama Padi, Jagung dan Kedelai MT 2017/2018 di Indonesia*. Balai Besar Peramalan Organisme Pengganggu Tumbuhan. https://berita.bbpopt.id/wp-content/uploads/2018/02/buku-evaluasi-mt-2017-dan-ramalan-mt-2017-2018.pdf

Beckerman, J. L. (2013). Detection of Fungicide Resistance. In *Fungicides - Showcases of Integrated Plant Disease Management from Around the World* (pp. 281–310). InTech. https://doi.org/10.5772/55981

Bock, C. H., Jeger, M. J., Mughogho, L. K., Cardwell, K. F., Mtisi, E., Kaula, G., & Mukansabimana, D. (2000). Variability of *Peronosclerospora sorghi* isolates from different geographic locations and hosts in Africa. *Mycological Research*, *104*(1), 61–68. https://doi.org/10.1017/S0953756299008965

Bonde, M. R. (1982). Epidemiology of downy mildew diseases of maize, sorghum and pearl millet. *Tropical Pest Management*, *28*(1), 49–60. https://doi.org/10.1080/09670878209370674

Bradley, C. A., Hollier, C., & Kelly, H. (2012). *Principles of Fungicide Resistance* (pp. 1–5). Fungicide Resistance Action Comitee (FRAC). https://www.plantmanagementnetwork.org/hub/SoyFungicideResistance/files/FungicideResistance.pdf

Burhanuddin. (2015). Masalah penyakit bulai dan alternatif pemecahannya di Kecamatan Pagu Kabupaten Kediri Propinsi Jawa Timur. *Prosiding Seminar Nasional Serealia*, 375–380. https://docplayer.info/111562191-Masalah-penyakit-bulai-dan-alternatif-pemecahannya-di-kecamatan-pagu-kabupaten-kediri-propinsi-jawa-timur.html

Chen, F., Zhou, Q., Xi, J., Li, D., Schnabel, G., & Zhan, J. (2018). Analysis of RPA190 revealed multiple positively selected mutations associated with metalaxyl resistance in Phytophthora infestans. *Pest Management Science*, *74*(8), 1916–1924. https://doi.org/10.1002/ps.4893

Chen, J., & Bo, X. (2019). *Pythium subutonaiense*, a new aquatic oomycete from Southern China based on morphological and molecular Characters. *Mycobiology*, *47*(3), 1–7. https://doi.org/10.1080/12298093.2019.1642700

Cohen, Y. (2015). The novel oomycide oxathiapiprolin inhibits all stages in the asexual life cycle of *Pseudoperonospora cubensis* - causal agent of cucurbit downy mildew. *PLoS ONE*, *10*(10), 1–22. https://doi.org/10.1371/journal.pone.0140015

Crouch, J. A., Davis, W. J., Shishkoff, N., Castroagudín, V. L., Martin, F., Michelmore, R., & Thines, M. (2022). *Peronosporaceae* species causing downy mildew diseases of Poaceae, including nomenclature revisions and diagnostic resources. *Fungal Systematics and Evolution*, *9*(June), 43–86. https://doi.org/10.3114/fuse.2022.09.05

Damicone, J. (2008). Fungicide resistance management. In *Oklahoma Cooperative Extension Fact Sheet* (Vol. 1, pp. 1–8). Oklahoma State University. http://pods.dasnr.okstate.edu/docushare/dsweb/Get/Version-6953/EPP-7663web.pdf

Department of Environmental Conservation. (2015). *Active Ingredient Data Package Metalaxyl and Mefenoxam* (Vol. 4). https://www.dec.ny.gov/docs/materials_minerals_pdf/mefenoxamdata.pdf

Eliestya, S., Anto, A., & Suriansyah. (2014). *Teknologi Budidaya Jagung dengan Pendekatan PTT* (R. Massinai (ed.); 1st ed.). Balai Pengkajian Teknologi Pertanian (BPTP). http://kalteng.litbang.pertanian.go.id/ind/images/data/buku-jagung-2014.pdf

Fisher, D. J., & Hayes, A. L. (1982). Mode of action of the systemic fungicides furalaxyl, metalaxyl and ofurace. *Pesticide Science*, *13*(3), 330–339. https://doi.org/10.1002/ps.2780130316

Fisher, D. J., & Hayes, A. L. (1984). Studies of mechanisms of metalaxyl fungitoxicity and resistance to metalaxyl. *Crop Protection*, *3*(2), 177–185. https://doi.org/10.1016/0261-2194(84)90052-8

Frederiksen, R. A. (1980). Sorghum downy mildew in the United States: Overview and Outlook. *Plant Disease*, *64*(10), 903–908. https://www.apsnet.org/publications/plantdisease/backissues/documents/1980articles/plantdisease64n10_903.pdf

Fungicide Resistance Action Comitee. (2017). *First meeting of the FRAC OSBPI working group*. http://www.frac.info/docs/default-source/osbpi-wg/minutes-of-the-2016-osbpi-wg-meeting-recommendations-for-2017---4apr17.pdf?sfvrsn=b8824a9a_2

Fungicide Resistance Action Comitee. (2018). FRAC Code List 2018: fungicide sorted by mode of action (including FRAC Code numbering). In *Frac Code List*. http://www.frac.info/docs/default-source/publications/frac-code-list/frac-code-list-2015-finalC2AD7AA36764.pdf?sfvrsn=4

Gao, R., & Zhang, G. (2013). Potential of DNA barcoding for detecting quarantine fungi. *Phytopathology*, *103*(11), 1103–1107. https://doi.org/10.1094/PHYTO-12-12-0321-R

Gisi, U., & Sierotzki, H. (2008). Fungicide modes of action and resistance in downy mildews. *European Journal of Plant Pathology*, *122*(1), 157–167. https://doi.org/10.1007/978-1-4020-8973-2_12

Gisi, U., & Sierotzki, H. (2015). Oomycete Fungicides: Phenylamides, Quinone Outside Inhibitors, and Carboxylic Acid Amides. In *Fungicide Resistance in Plant Pathogens* (pp. 145–174). Springer Japan. https://doi.org/10.1007/978-4-431-55642-8

Hamilton, D. (2002). *Metalaxyl-m (212)* (Issue 212). http://www.fao.org/fileadmin/templates/agphome/documents/Pests_Pesticides/JMPR/Evaluation04/MetalaxylM.pdf

Hikmawati, Kuswinanti, T., Melina, & Pabendon, M. B. (2011). Karakterisasi morfologi *Peronosclerospora* spp., penyebab penyakit bulai pada tanaman jagung, dari beberapa daerah di Indonesia. *Jurnal Fitomedika*, *7*(3), 159–161. https://www.semanticscholar.org/paper/Karakterisasi-Morfologi-Peronosclerospora-spp.%2C-di-Hikmahwati-Kuswinanti/f05fe767fc17626ba356b0a3b9b8f5b0255905c2

Hobbelen, P. H. F., Peveley, N. D., & Bosch, F. (2014). The emergence of resistance to fungicides. *PLoS ONE*, *9*(3), 1–14. https://doi.org/10.1371/journal.pone.0091910

Hudspeth, D. S. ., Nadler, S. A., & Hudspeth, M. E. S. (2000). A COX2 molecular phylogeny of the Peronosporomycetes. *Mycologia*, *92*(4), 674–684. https://doi.org/10.2307/3761425

Ishii, H. (2006). Impact of fungicide resistance in plant pathogens on crop disease control and agricultural environment. *Japan Agricultural Research Quarterly*, *40*(3), 205–211. https://doi.org/10.6090/jarq.40.205

Kammarnjesadakul, P., Palaga, T., Sritunyalucksana, K., Mendoza, L., Krajaejun, T., Vanittanakom, N., Tongchusak, S., Denduangboripant, J., & Chindamporn, A. (2011). Phylogenetic analysis of *Pythium insidiosum* Thai strains using cytochrome oxidase II (COX II) DNA coding sequences and internal transcribed spacer regions (ITS). *Medical Mycology*, *49*(3), 289–295. https://doi.org/10.3109/13693786.2010.511282

Kughur, P. G., & Audu, O. (2015). Effects of intensive agricultural production on the environment in Benue State, Nigeria. *IOSR Journal of Agriculture and Veterinary Science Ver. I*, *8*(8), 7–11. https://doi.org/10.9790/2380-08810711

Leon, C. D. (2004). *Maize Diseases: a guide for field identification* (1st ed.). CIMMYT. https://repository.cimmyt.org/bitstream/handle/10883/3707/13180.pdf?sequence=1&isAllowed=y

Lukman, R., Afifuddin, A., & Lubberstedt, T. (2012). Unraveling the genetic diversity of maize downy mildew in Indonesia. *Journal of Plant Pathology & Microbiology*, *04*(02), 2–9. https://doi.org/10.4172/2157-7471.1000162

Mathiyazhagan, S., Karthikeyan, M., Sandosskumar, R., & Velazhahan, R. (2008). Analysis of variability among the isolates of *Peronosclerospora sorghi* from sorghum and cornbased on restriction fragment length polymorphismof ITS region of ribosomal DNA. *Archives of Phytopathology and Plant Protection*, *41*(1), 31–37. https://doi.org/10.1080/03235400600628062

Muis, A., Nonci, N., & Pabendon, M. B. (2016). Geographical distribution of *Peronosclerospora* spp., the causal organism of maize downy mildew, in Indonesia. *AAB Bioflux*, *8*(3), 143–155. http://www.aab.bioflux.com.ro/docs/2016.143-155.pdf

Muis, A., Suriani, Kalqunty, S. H., & Nonci, N. (2018). *Penyakit Bulai pada Tanaman Jagung dan Upaya Pengendaliannya* (1st ed.). Penerbit Deepublish. https://www.researchgate.net/publication/329363769_Penyakit_Bulai_Pada_Tanaman_Jagung_dan_Upaya_Pengendaliannya

Muis, Amran, Ryley, M. J., Tan, Y. P., Suharjo, R., Nonci, N., Danaatmadja, Y., Hidayat, I., Widiastuti, A., Widinugraheni, S., Shivas, R. G., & Thines, M. (2023). *Peronosclerospora neglecta* sp. nov.—a widespread and overlooked threat to corn (maize) production in the tropics. *Mycological Progress*, *22*(2), 1–7. https://doi.org/10.1007/s11557-022-01862-5

Pasteris, R. J., Hanagan, M. A., Bisaha, J. J., Finkelstein, B. L., Hoffman, L. E., Gregory, V., Shepherd, C. P., Andreassi, J. L., Sweigard, J. A., Klyashchitsky, B. A., Henry, Y. T., & Berger, R. A. (2015). The discovery of oxathiapiprolin: A new, highly-active oomycete fungicide with a novel site of action. In *ACS Symposium Series* (1st ed., Vol. 1204, pp. 149–161). American Chemical Society. https://doi.org/10.1021/bk-2015-1204.ch011

Pusdatin. (2017). *Outlook Tanaman Pangan dan Hortikultura 2017*. Pusat Data dan Sistem Informasi Pertanian. http://epublikasi.setjen.pertanian.go.id/epublikasi/outlook/2017/Outlook TPHORTI 2017/files/assets/basic-html/page22.html

Randall, E., Young, V., Sierotzki, H., Scalliet, G., Birch, P. R. J., Cooke, D. E. L., Csukai, M., & Whisson, S. . (2014). Sequence diversity in the large subunit of RNA polymerase I contributes to Mefenoxam insensitivity in *Phytophthora infestans*. *Molecular Plant Pathology*, *15*(7), 664–676. https://doi.org/10.1111/mpp.12124

Rashid, Z., Zaidi, P. H., Vinayan, M. T., Sharma, S. S., & Setty, T. A. S. (2013). Downy mildew resistance in maize (Zea mays L.) across *Peronosclerospora* species in lowland tropical Asia. *Crop Protection*, *43*(2013), 183–191. https://doi.org/10.1016/j.cropro.2012.08.007

Robideau, G. P., Cock, A. W. A. M., Coffey, M. D., Voglmayr, H., Brouwer, H., Bala, K., Chitty, D. W., Desaulniers, N., Eggerston, Q. A., Gachon, C. M. M., Hu, C. H., Kupper, F. C., Rintoul, T. L., Sarhan, E., Verstappen, E. C. P., Zhang, Y., Bonants, P. J. M., Ristaino, J. B., & Levesque, C. A. (2011). DNA barcoding of oomycetes with cytochrome c oxidase subunit I and internal transcribed spacer. *Molecular Ecology Resources*, *11*(6), 1002–1011. https://doi.org/10.1111/j.1755-0998.2011.03041.x

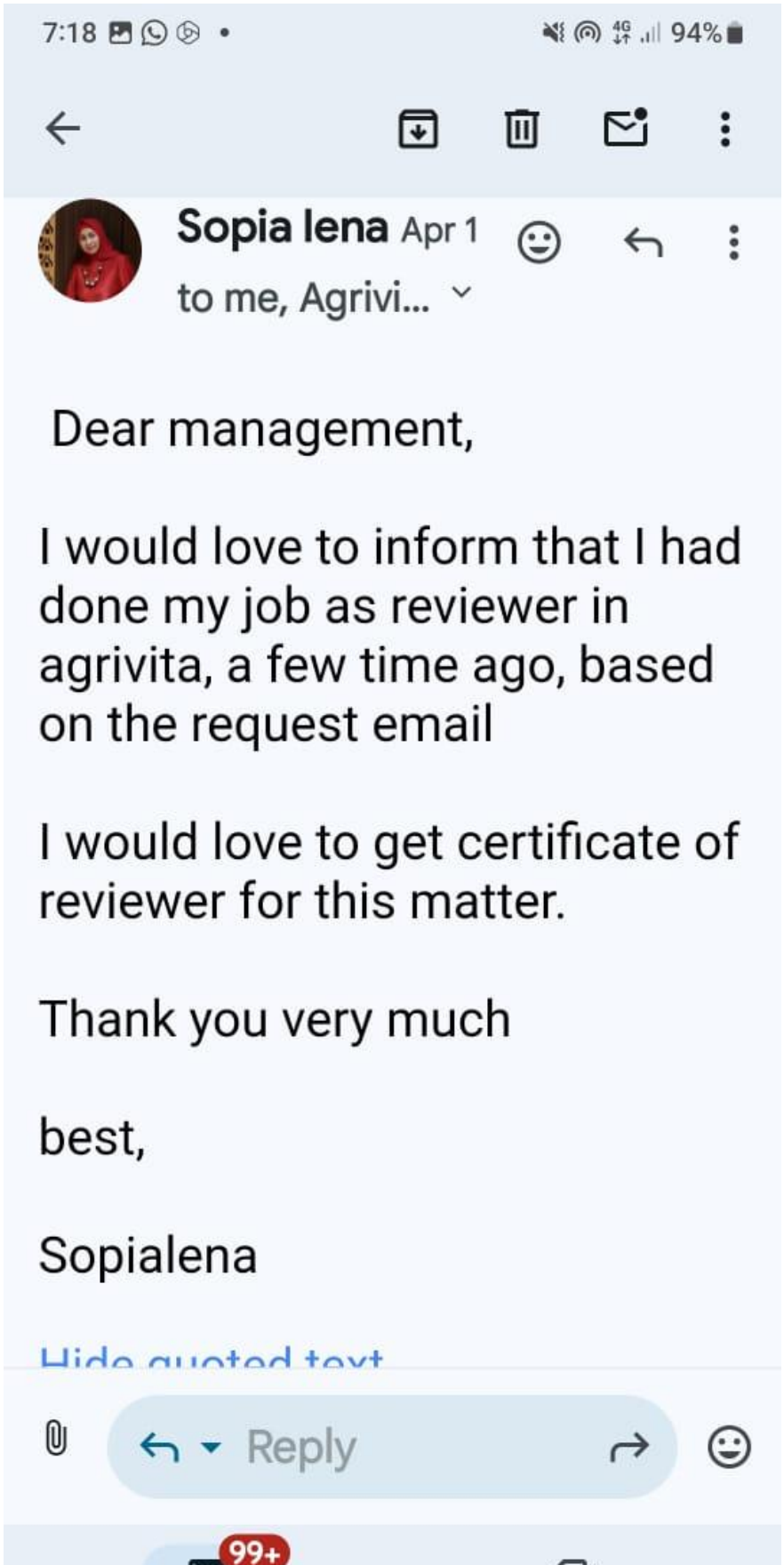
Rustiani, U. S. (2015). Keragaman dan Pemetaan Penyebab Penyakit Bulai Jagung di 13 Provinsi Indonesia [Institut Pertanian Bogor]. In *Repository Intitut Pertanian Bogor*. https://repository.ipb.ac.id/handle/123456789/77406

Rustiani, U. S., Sinaga, M. S., Hidayat, H., & Wiyono, S. (2015a). Ecological characteristic of Peronosclerospora maydis in Java, Indonesia. *International Journal of Science*, *19*(1), 159–167. https://gssrr.org/index.php/JournalOfBasicAndApplied/article/view/3191/2047

Rustiani, U. S., Sinaga, M. S., Hidayat, S. H., & Wiyono, S. (2015b). Tiga spesies *Peronosclerospora* penyebab penyakit bulai jagung di Indonesia. *Berita Biologi*, *14*(1), 29–37. https://e-journal.biologi.lipi.go.id/index.php/berita_biologi/article/view/1860/1745

Sierotzki, H., Quaranta, L., Müller, U., & Gisi, U. (2019). Nucleic Acid Synthesis Inhibitors: Metalaxyl-M. In P. Jeschke, M. Witschel, W. Kramer, & U. Schrimer (Eds.), *Modern Crop Protection Compounds* (3rd ed., pp. 949–958). Wiley-VCH Verlag GmbH & Co. KGaA.

https://doi.org/10.1002/9783527699261.ch25
Singh, S. ., Bal, S., & Thakur, D. P. (1996). Problems and strategies in the control of downy mildew. *Proceedings of the International Pearl Millet Workshop*, 1–34. https://oar.icrisat.org/4436/1/CP_377.pdf
Studholme, D. J., Panda, P., Stowasser, E. S. V., Gomzalez, M., Hill, R., Sambles, C., Grant, M., Williams, N. M., & McDougal, R. L. (2019). Genome sequencing of oomycete isolates from Chile supports the New Zealand origin of *Phytophthora kernoviae* and makes available the first Nothophytophthora sp. genome. *Molecular Plant Pathology*, *20*(3), 423–431. https://doi.org/10.1111/mpp.12765
The CIMMYT Maize Program. (2004). *Maize diseases: a guide for field identification* (4th editio). CIMMYT. https://repository.cimmyt.org/xmlui/bitstream/handle/10883/775/78507.pdf?sequence=4&isAllowed=y
Toffolatti, S. L., Prandato, M., Serrati, L., Sierotzki, H., Gisi, U., & Vercessi, A. (2011). Evolution of Qol resistance in Plasmopara viticola oospores. *European Journal of Plant Pathology*, *129*(2), 331–338. https://doi.org/10.1007/s10658-010-9677-y
Waceke, J. W., & Kimenju, J. . W. (2007). Intensive subsistence agriculture: impacts, challenges and possible interventions. *Dynamic Soil, Dynamic Plant*, *1*(1), 43–53. http://www.globalsciencebooks.info/Online/GSBOnline/images/0706/DSDP_1(1)/DSDP_1(1)43-53o.pdf
Widiantini, F., Pitaloka, D. J., Nasahi, C., & Yulia, E. (2017). Perkecambahan *Peronosclerospora* spp. asal beberapa daerah di Jawa Barat pada fungisida berbahan aktiff metalaksil, dimetomorf, dan fenamidon. *Jurnal Agrikultura*, *28*(2), 95–102. https://doi.org/https://doi.org/10.24198/agrikultura.v28i2.15753
Widiantini, F., Yulia, E., & Purnama, T. (2015). Morphological variation of *Peronosclerospora maydis*, the causal agent of maize downy mildew from different locations in Java-Indonesia. *Journal of Agricultural Engineering and Biotechnology*, *3*(2), 23–27. https://doi.org/10.18005/JAEB0302002
Yang, C., Hamel, C., Vujanovic, V., & Gan, Y. (2011). Fungicide: modes of action and possible impact on nontarget microorganisms. *ISRN Ecology*, *2011*, 1–8. https://doi.org/10.5402/2011/130289
Zubachtirodin, Saenong, S., Pabbage, M. S., Azrai, M., Setyorini, D., Kartaatmadja, S., & Kasim, F. (2016). *Pedoman Umum PTT Jagung* (3rd ed.). Pusat Penelitian dan Pengembangan Tanaman Pangan. http://pangan.litbang.pertanian.go.id/files/pedumpajale/pttjagung.pdf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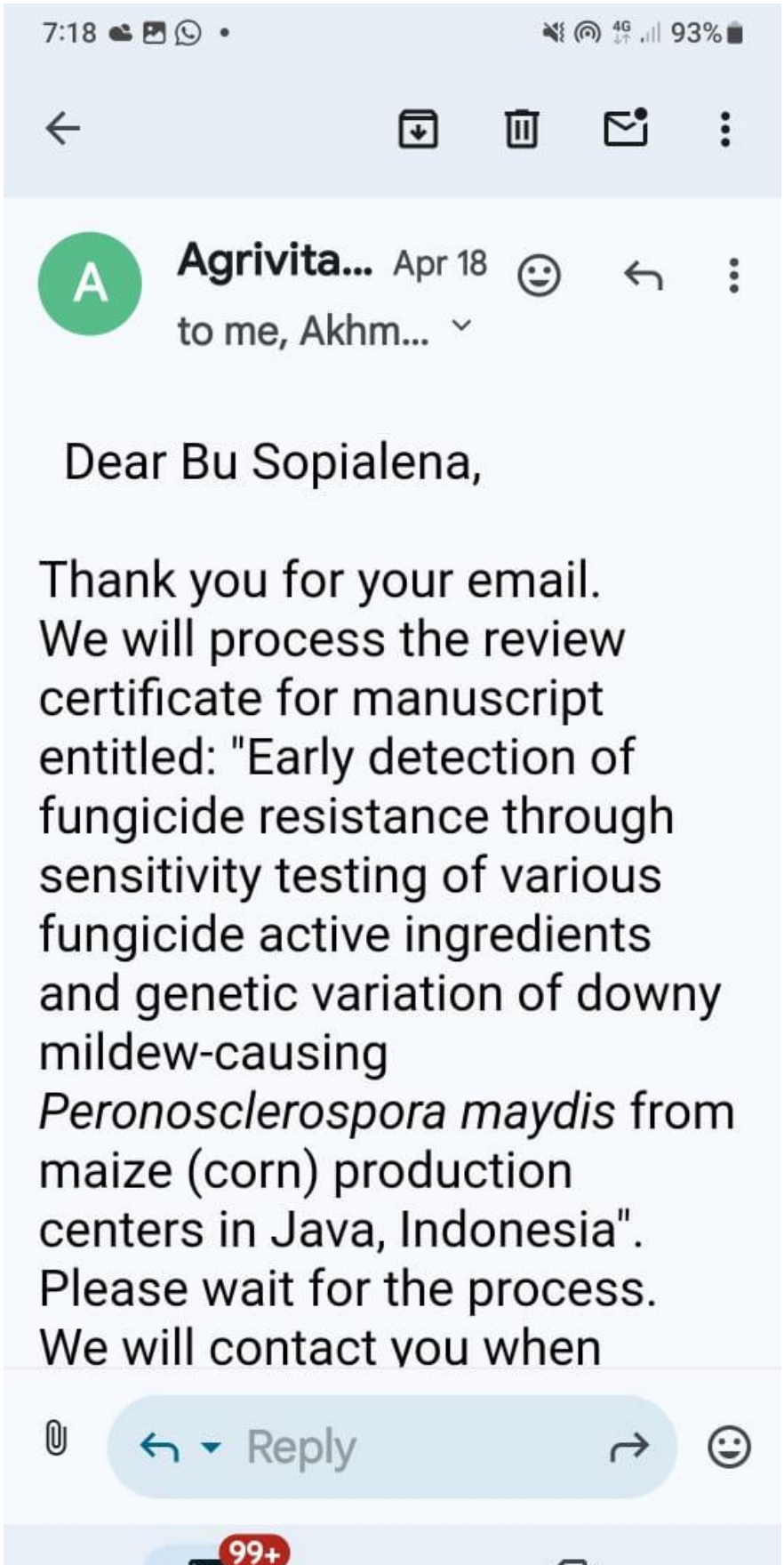
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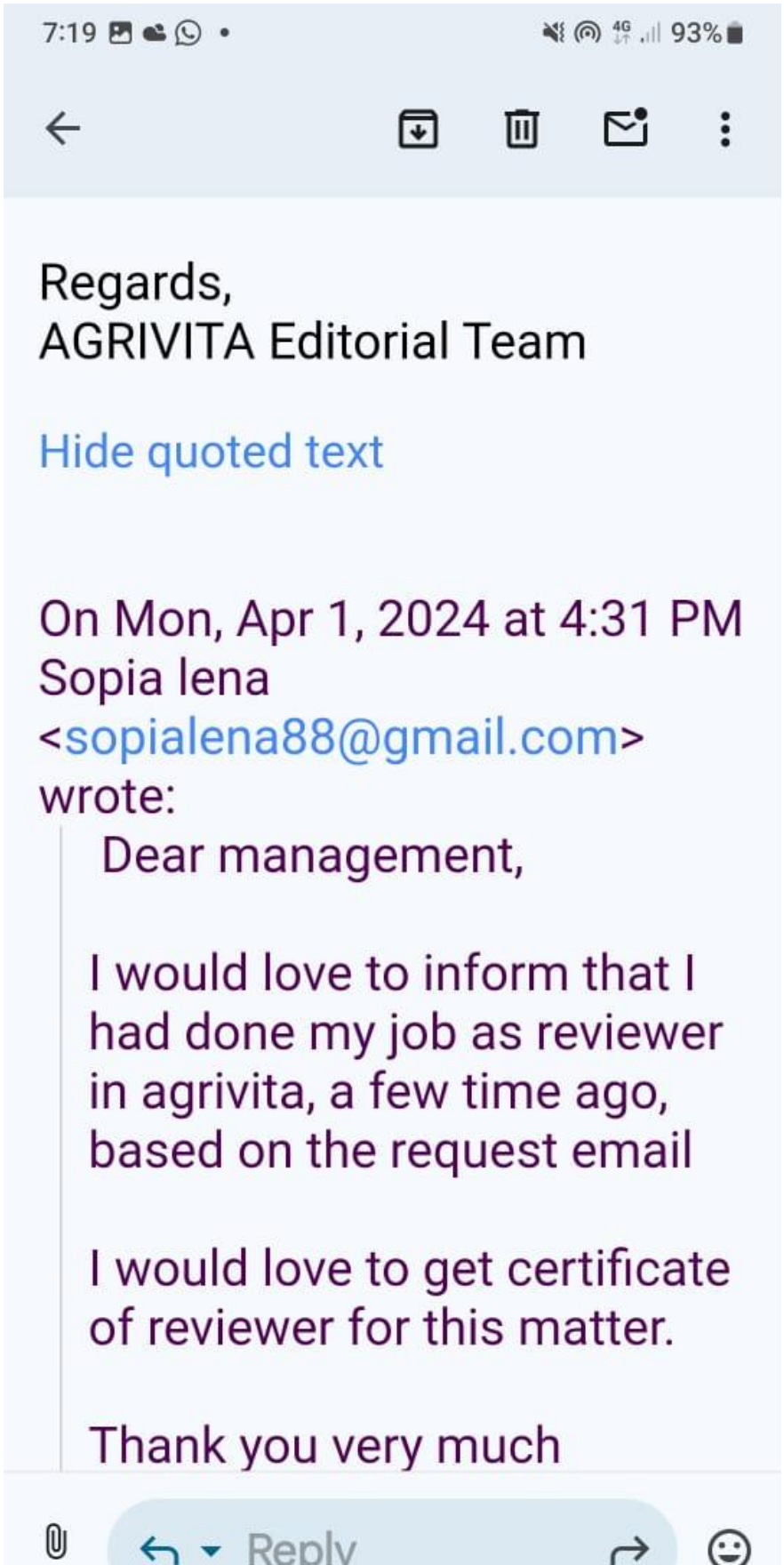
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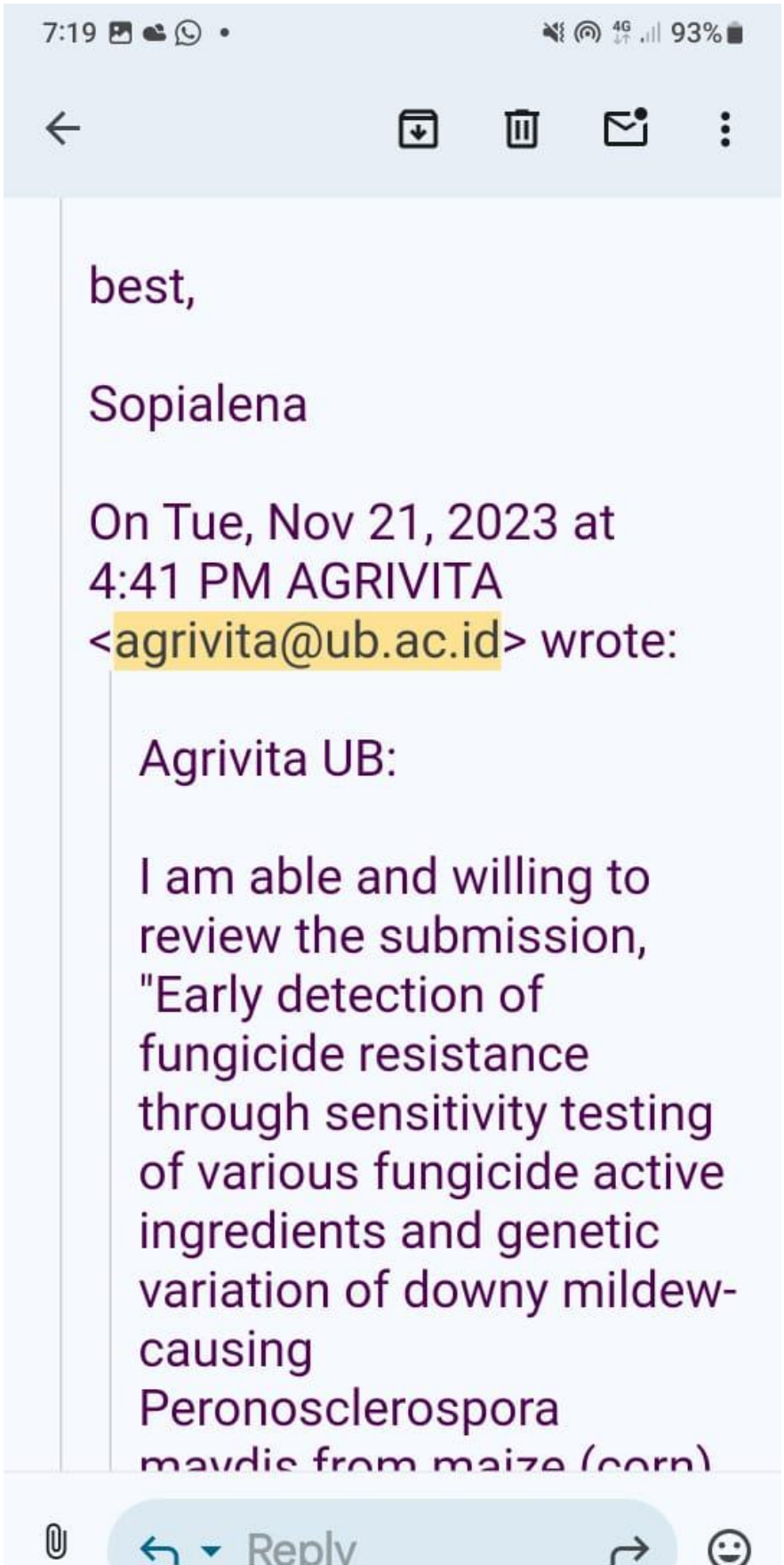
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