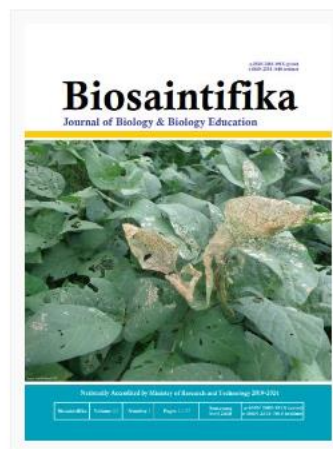




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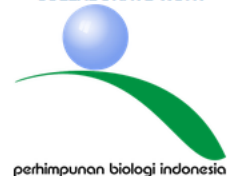
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Abstract

Soybean (*Glycine max* L. Merr.) is the third food crop commodity after rice and maize in Indonesia. This plant is also known as the most important source of vegetable protein, which is relatively inexpensive, but a decrease in soybean productivity can occur due to infection with disease-causing pathogens, one of is *Cercospora kikuchii* which causes Cercospora leaf blight (CLB). The research objectives were to determine the anatomical resistance and disease severity of soybean cultivars against CLB. The method was an experiment with a completely randomized design (CRD) factorial pattern; factor 1 being soybean cultivars (Dering, Slamet, Grobogan, Wilis) and factor 2, namely pathogen inoculation (0 conidiospores/mL and 10^5 conidiospores/mL). Anatomical method preparations using paraffin, staining with 1% safranin. Disease criteria are based on the council of scientific and industrial research (CSIR) assessment method. Data were analysis used analysis of variance ($p < 0.05$) and the least significance difference (LSD). The results showed that Dering and Slamet cultivars had the largest cuticle, epidermis, and palisade ratios and the smallest stomata length and width with the largest number of stomata and trichomes compared to Grobogan and Wilis. The disease severity (DS) of the cultivars Dering 14.6%, Slamet 24.64%, Grobogan 24.80% (classified as a resistant with low infection), while Wilis cultivar was 31.08% as a moderately susceptible cultivar with moderate infection. The novelty of soybean cultivar selection against CLB is important and its effectiveness for increasing soybean productivity. Dering, Slamet and Grobogan are likely to be further developed with their resistance to CLB disease.

Keywords

Cercospora kikuchii, cultivar selection, fungal pathogen, *Glycine max*, plant disease

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Soybean Selection Against *Cercospora* Leaf Blight Disease Caused By *Cercospora kikuchii* Based on Anatomical Resistance

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Abstract. Soybean (*Glycine max* L. Merr.) is the third food crop commodity after rice and maize in Indonesia. This plant is also known as the most important source of vegetable protein, which is relatively inexpensive, but a decrease in soybean productivity can occur due to infection with disease-causing pathogens, one of is *Cercospora kikuchii* which causes Cercospora leaf blight (CLB). The research objectives were to determine the anatomical resistance and disease severity of soybean cultivars against CLB. The method was an experiment with a completely randomized design (CRD) factorial pattern; factor 1 being soybean cultivars (Dering, Slamet, Grobogan, Wilis) and factor 2, namely pathogen inoculation (0 conidiospores/mL and 10⁵ conidiospores/mL). Anatomical method preparations using paraffin, staining with 1% safranin. Disease criteria are based on the council of scientific and industrial research (CSIR) assessment method. Data were analysis used analysis of variance ($p < 0.05$) and the least significance difference (LSD). The results showed that Dering and Slamet cultivars had the largest cuticle, epidermis, and palisade ratios and the smallest stomata length and width with the largest number of stomata and trichomes compared to Grobogan and Wilis. The disease severity (DS) of the cultivars Dering 14.6%, Slamet 24.64%, Grobogan 24.80% (classified as a resistant with low infection), while Wilis cultivar was 31.08% as a moderately susceptible cultivar with moderate infection. The novelty of soybean cultivar selection against CLB is important and its effectiveness for increasing soybean productivity. Dering, Slamet and Grobogan are likely to be further developed with their resistance to CLB disease.

Key words: *Cercospora kikuchii*, cultivar selection, fungal pathogen, *Glycine max*, plant disease

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INTRODUCTION

National soybean production is still low, which is only 1.1 tons ha⁻¹. This productivity can still be increased again to 1.5-2.5 tons ha⁻¹, with the application of advanced technology and more intensive cultivation systems. Although national soybean production in the last five years has increased by an average of 2.49% per year, nationally the increase in soybean production for the 2011-2015 period has only been realized in 2014 by 22.44% and 2015 by 4.59%, while the previous three years it decreased. by 6.15% (2011), 0.96% (2012), and 7.49% (2013). However, soybean production in Java and Outside Java was also reduced. Java Island in 2013 decreased by 13.53% and Outside Java in 2012

decreased by 13.59% (Nuryati et al., 2015). The highest decline in soybean productivity with 8.92% occurred in 2012 (Hermanto et al., 2019). One of the causes of low production is soybean disease caused by fungal pathogen. The most common plant diseases in tropical regions like Indonesia is the leaf blight caused by the *C. kikuchii* or known as Cercospora leaf blight (CLB). The fungus attacks the leaves, and the leaves die quickly and fall off. *C. kikuchii* also infect the seeds and damages the seeds marked with purple color (de Mello et al., 2021).

C. kikuchii causes dark purple lesions on leaves, petioles, and premature defoliation. This disease reduces soybean production with leaf loss

in the later stages of plant development (Kashiwa et al., 2021). Symptoms of leaf blight due to infection with *C. kikuchii* showed characteristic dark purple lesions on the leaves and petiole. This is caused by cercosporin, which is a pigment produced by pathogens. The pigment induces host plant cell death (Kashiwa & Suzuki, 2021). In addition, the fungus *C. kikuchii* that attacks the leaves and causes CLB disease will change the leaf structure both anatomically and morphologically also early defoliation with necrotic (Poletto et al., 2021). As a result of CLB, the photosynthetic function will be disrupted, which results in plants lacking a source of photosynthate needed for metabolism. This is one of the causes of reduced soybean production. Therefore, supporting information and knowledge are needed in an effort to increase soybean production, one of which is to develop cultivars that are resistant to CLB disease. Resistant cultivars tend to have a thicker cuticle and epidermis with dense cells, few stomata and a lot of trichomes while susceptible cultivars tend to have a thin cuticle layer and few trichomes, so that the leaf anatomy characteristics of each soybean cultivar can be compared its resistance to *C. kikuchii*. Smaller stomatal density can minimizing infection from pathogens (Qi et al., 2018). Resistant cultivars tend to have thick epidermis, thick cuticle layer with high cellulose content, to increase resistance to pathogen infection (Hemati et al., 2018).

Leaves are one of the initial sites of deposition of spores of the pathogenic fungus *C. kikuchii* before infecting. The stomata (pores) can be an entry point for the haustorium hyphae and appressorium of the fungus. A large number of stomata and wide pores make it easier for pathogenic fungi to infect, whereas the small pores can prevent fungal hyphae from entering. Leaf structure starting from the cuticle, epidermis, and stomata is the initial defense of plants to prevent pathogen infection, then biochemically such as quinone is the next defense of plants to prevent pathogen infection. Previous studies have shown that epidermal cells in disease-resistant cultivars can better minimize infection with *Macrophomina phaseolina* preventing hyphae from penetrating 6 days after inoculation compared to susceptible cultivars (Hemati et al., 2018). This histopathological evaluation and investigation is very important to do to find out more about the interaction between the pathogen and the resistance of soybean cultivars. Histopathological observations can provide information and an

overview of the effects of pathogenic infections and their contribution to disease development (Yeung et al., 2015).

Research related to the evaluation and investigation of the resistance of soybean cultivars to *C. kikuchii* infection that causes CLB based on the anatomical structure has never been carried out, so it is necessary to do so especially on commercial soybean commodities that are widely cultivated in Indonesia, namely the Dering, Slamet, Grobogan and Wilis cultivars. This study aimed to evaluate and determine the characteristics and anatomical differences of several soybean cultivars against *C. kikuchii* fungal infection and to determine the level of resistance of these soybean cultivars from Cercospora leaf blight caused by *C. kikuchii* infection based on disease severity values.

METHODS

Plant materials, fungal pathogens, and experimental methods

The study used 4 soybean cultivars, consisting of 2 disease-resistant cultivars (Dering and Slamet), and 2 disease-prone cultivars (Grobogan and Wilis). The seeds were obtained in fresh condition after harvest from the soybean plant breeding laboratory, Faculty of Agriculture, Jenderal Sudirman University. Cercospora kikuchii isolates were obtained from the Indonesian Culture Collection (InaCC) of the Indonesian Institute of Sciences (LIPI), Bogor with isolate number InaCC F177 in February 2017 in the form of cultures in petri dishes and test tubes. The study used an experimental method with a completely randomized design (CRD) with a factorial pattern (two factors). The first factor was soybean cultivar which consisted of four cultivars, namely Dering (G1), Slamet (G2), Grobogan (G3), and Wilis (G4). The second factor was *C. kikuchii* inoculation of 0 conidiospores/mL (I0), and 10⁵ conidiospores/mL (I1), with 5 replications. Observations of soybeans were carried out after planting the seeds or after the third leaf appeared.

Preparation of conidiospores suspension and observation of incubation period

The conidiospores liquid suspension was made from the rejuvenation of the fungal isolate *C. kikuchii* (InaCC F177) on slanted media aged 14 days after incubation, then 10 mL of sterile distilled water was added. *C. kikuchii* conidiospores inoculation was carried out once, namely when soybean plants began to grow at least the 3rd leaf.

Inoculation was carried out in the afternoon, and each soybean plant was inoculated with 10 mL conidiospores suspension of *C. kikuchii*. Inoculation was carried out by spraying on the upper and lower leaf surfaces using a sprayer, with a conidia density of 10^5 conidiospores/mL, which was calculated using a hemocytometer. Observation of the incubation period for each cultivar was carried out every day to obtain data on the incubation period (until the first symptoms of *Cercospora* leaf blight appear) (Leucker et al., 2017).

Observation of the severity of *Cercospora* leaf blight

Observation of disease severity was carried out after one day of inoculation of *C. kikuchii* inoculum until the end of the soybean vegetative period (20 days) (Isnawan & Mubarak, 2014). Calculation of Disease Severity (DS) that appears on the leaves is based on the category:

0 = asymptomatic

1= leaf blight on leaves 0.1 cm in diameter $0 < X < 10\%$

2= leaf blight on leaves 0.5 cm in diameter $0 < X < 30\%$

3= blight on leaves 1.0 cm in diameter $0 < X < 50\%$

4= leaf blight on leaves 1.0-2.0 cm in diameter more than 50%

Disease Severity (DS) is then calculated by the equation (Cahyaningrum et al., 2020):

$$DS = \frac{\sum(n \times v)}{Z \times N} \times 100\%$$

Description: DS (disease severity), n (number of leaves with blight symptoms per attack category), v (scale value for each attack category) Z (highest category scale value for attack category), N (number of leaves observed).

The results of the calculation of disease severity were then categorized based on the criteria for plant resistance to disease infection based on the value of disease severity based on the modified CSIR method that modified (Table 1) (Wongnaa et al., 2021).

Observation of stomata size, stomatal density and trichomes

Observation of stomata size (length and width) was carried out using a micrometer mounted on calibrated ocular and objective lenses. Calculation of the density of stomata and trichomes was carried out by performing paradermal incisions on the leaves. Making fresh preparations includes (1) applying nail polish to the abaxial and adaxial surfaces of soybean leaves, then waiting 15-20 minutes, (2) after the nail polish dries, then carefully transferred and placed on the object-glass (3) observations are made with a light microscope at 400x magnification (Galdon-Armero et al., 2018). Calculation of the density of stomata and trichomes follows the following formula:

$$D = \frac{TNS / TNT}{mm^2 \text{ of the field view}}$$

Description: D: Density, TNS / TNT: Total Number of Stomata/ Trichomes

Observation of leaf anatomy by embedding method

Observation of leaf anatomy by making preparations using the embedding method was carried out to observe and measure cuticle thickness, abaxial and adaxial epidermis thickness, palisade ratio, and leaf mesophyll thickness. The sample used was the 5th leaf from the top of the plant, then it was cut to a size of 1 cm². The embedding method for the preparation of the preparations included: (1) fixation, leaf samples were placed in FAA solution with a composition of 10% formalin, 5% acetic acid, 50% ethyl alcohol, and 35% distilled water) for 24 hours, (2) staining using safranin 1% in 70% alcohol, (3) dehydration using ethyl alcohol, (4) dealcoholization using xylol solution, (5) embedding using paraffin, (6) then sectioning step using a manual rotary microtome (0.5-60 µm Nahita) transversely. Observation and measurement of the thickness of the cuticle, epidermis, and mesophyll using a calibrated micrometer, while the palisade ratio was measured by cells per area of field of view (mm²), using a light microscope (Olympus CH-20) at 400x magnification (Yeung et al., 2015).

Table 1. Soybean resistance criteria against infectious diseases based on disease severity

Score	Criteria	Category Infections (%) covered by lesion	Description
1.0 – 1.99	Stand (S)	0	No infection no visible symptoms
2.0 – 2.99	Resistant (R)	$1 \leq DS \leq 25$	Low infection criteria 1–25% of total leaf area is covered by lesions and blight
3.0 – 3.99	Moderate Resistant (MR)	$26 \leq DS \leq 50$	Moderate infection criteria 26–50% of the total leaf area is covered by blight and necrotic, with 15% defoliation
4.0 – 4.99	Moderate Susceptible (RS)	$51 \leq DS \leq 75$	Severe infection criteria 51–75% of the total leaf area of the whole plant covered by blight and necrotic and more than 30% defoliation was occurred
5.0 – more	Susceptible (S)	$76 \leq DS \leq 100$	Susceptible criteria with 76–100% of the leaf area of the whole plant is blight and more than 50% of the total number of leaves is necrotic and defoliated

Source: Wongnaa et al. (2021) Modified; DS: Disease Severity

Data analysis

Data obtained from observations of leaf anatomical structures were analyzed using analysis of variance (ANOVA), which was further tested with the least significance difference (LSD) at a 5% confidence level using SPSS software version (25.0). Research data is presented in the form of a histogram (Husen et al., 2021).

RESULT AND DISCUSSION

Quantitative measurements of the leaf anatomy of four soybean cultivars showed a relationship between soybean leaf resistance and CLB resistance caused by *C. kikuchii*. The ability of the fungus *C. kikuchii* to infect soybean leaves and cause blight in each cultivar showed differences.

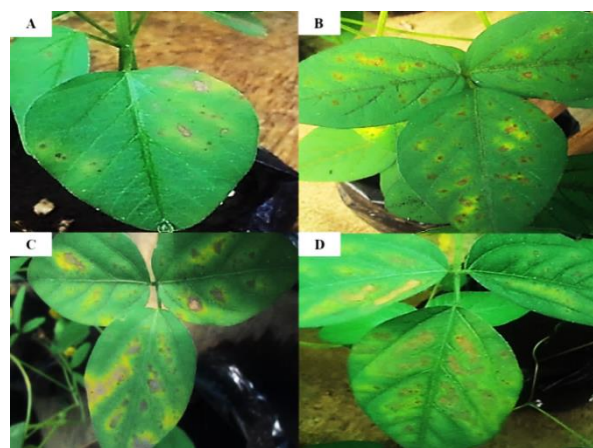


Figure 1. Fungal infection of *C. kikuchii* (Cercospora Leaf Blight) in 4 soybean cultivars (A) Dering; (B) Slamet; (C) Grobogan; (D) Willis

Genetic, biochemical, and leaf anatomical structures allow for these differences. Based on the results of the study, the leaves with the most blight and spot symptoms were the Wilis cultivar, while the cultivar with the least blight symptoms was the Slamet cultivar. The Slamet and Grobogan cultivars also showed blight, but not as much as the Wilis cultivar. The infection appears as dark brown lesions with brownish yellow, leaf lesions measuring about 0.2–3.5 cm with leaf margins beginning to dry, and necrosis (Figure 1). Based on observations of the incubation period of the fungus *C. kikuchii* on four cultivars, it was shown that the average incubation period of *C. kikuchii* was 10 days.

Symptoms of CLB appear as purplish leaves at the beginning of seed formation. Necrotic lesions develop on the upper leaves of the crown and then progress to the lower leaves, stems, and petioles (Albu et al., 2016). The results of the cuticle thickness measurement showed that there was a significant difference between the inoculated and uninoculated cultivars ($p < 0.05$). The highest cuticle thickness in the uninoculated cultivar was Slamet cultivar with 4.9 μm , while the lowest was Dering and Grobogan cultivar with 2.9 μm . The highest cuticle thickness in the inoculated group was the Dering cultivar with 4 m and the lowest was the Wilis cultivar with 2.8 μm (Figure 2).

The cuticle is one of the structures that role as a protector from pathogens. The cuticle also functions as an initial defense against pathogens into the leaves, and prevents the penetration of

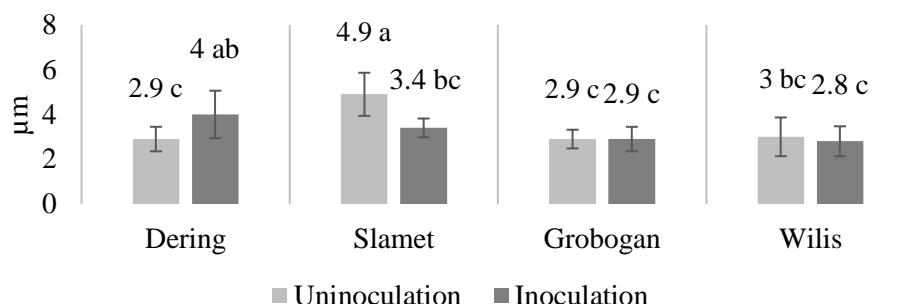


Figure 2. Cuticle thickness of 4 soybean cultivars

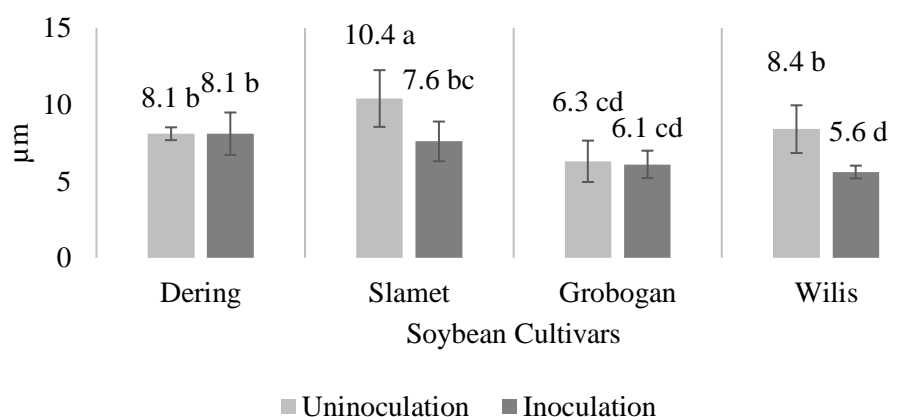


Figure 3. Epidermal thickness (adaxial) of 4 soybean cultivars

hyphae. The cuticle function related to humidity, when the humidity limit is below normal for the germination of pathogenic fungal spores, infection and pathogen penetration can be minimized or even prevented (Ortiz-urquiza & Keyhani, 2013). Pathogen infection can cause damage to the cuticle structure of leaves, but over time adaptation and physiological responses can cause the cuticle and leaf layers to become succulent and thickened (Chen et al., 2021). The attack of fungal pathogens can change anatomical structures such as cell size, changes in cell number, and hyperplasia (Reusche et al., 2012). Infection of *P. pachyrhizi* against Slamet soybean cultivars showed an increase in cuticle thickness of 21.43% (Samiyarsih et al., 2020).

The results of the measurement of the upper epidermis thickness (adaxial) showed a significant difference ($p < 0.05$). The highest upper epidermis thickness in the uninoculated group was Slamet cultivar with 10.4 µm and the lowest was the Grobogan cultivar with 6.3 µm. Meanwhile, the Slamet cultivar in inoculated group also had the highest thickness of the upper epidermis at 7.6 µm, and the Wilis cultivar had the lowest thickness with 5.4 µm (Figure 3).

The results of measuring the thickness of the lower epidermis (abaxial) showed a significant difference ($p < 0.05$). The highest thickness of the lower epidermis in the uninoculated group was the Slamet cultivar and the lowest was the Grobogan cultivar. Meanwhile, in the inoculated group, the Slamet cultivar also had the highest thickness of the lower epidermis, and the Grobogan cultivar had the lowest thickness of the upper epidermis (Figure 4).

Mesophyll thickness measurement results of four cultivars showed a very significant value ($p < 0.05$). Mesophyll thickness in the uninoculated group showed that Slamet cultivar had the highest mesophyll thickness, while in the inoculated group the highest mesophyll thickness was Dering (Figure 5).

Differences in mesophyll size in soybean cultivars can be influenced by genetic factors for each cultivar, optimization of photosynthetic reactions, or can occur due to lack of cell nutrition in mesophyll tissue due to pathogenic infection (Júnior et al., 2017).

The results of the palisade ratio measurement showed a significant difference ($p < 0.05$). The highest palisade ratio in the uninoculated group

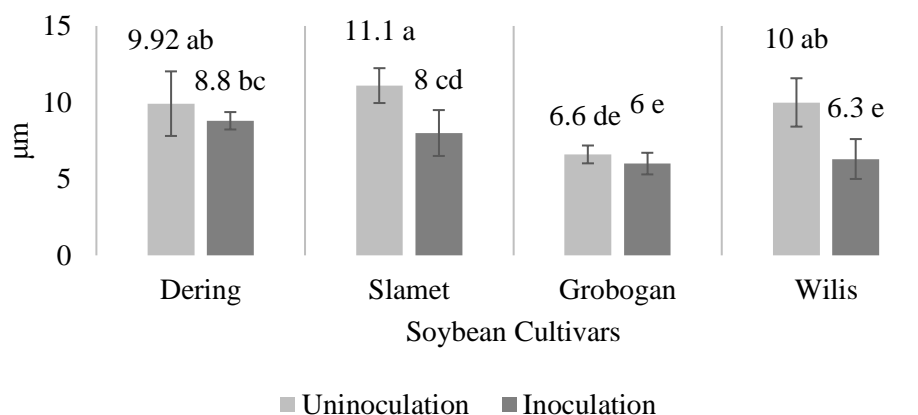


Figure 4. Epidermal thickness (abaxial) of leaves of 4 soybean cultivars

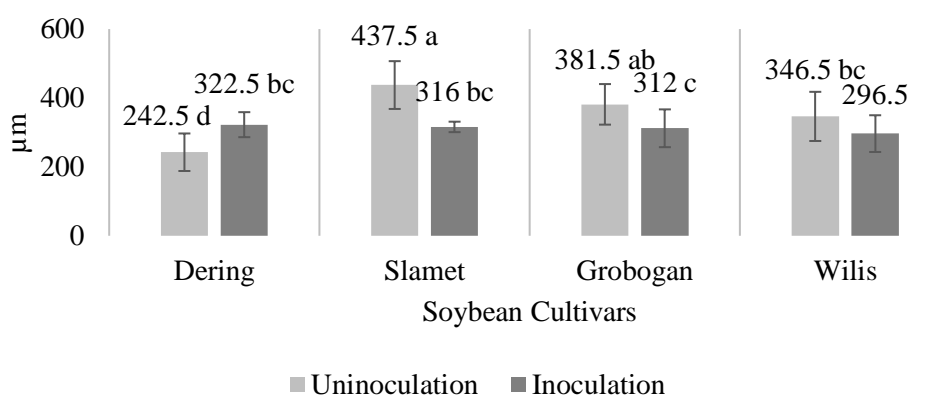


Figure 5. Leaf mesophyll thickness of 4 soybean cultivars

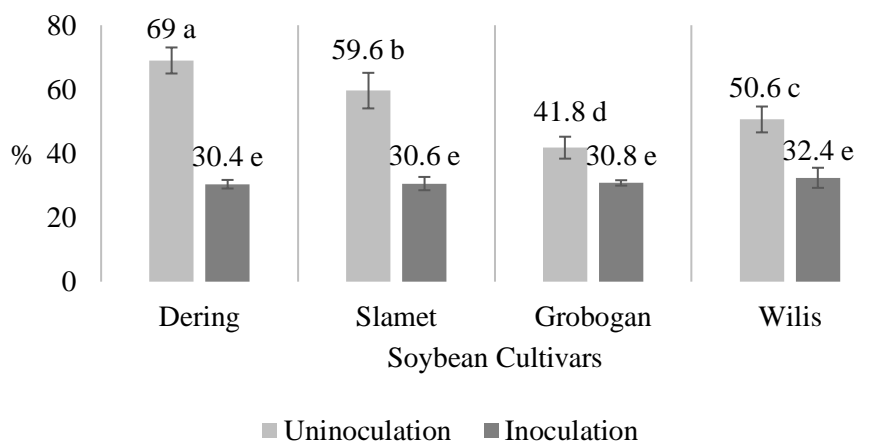


Figure 6. Palisade ratio of 4 soybean cultivars

was Dering cultivar with 69%, while the lowest was Grobogan cultivar with 41.8%. Meanwhile, for the inoculated cultivars, the highest palisade ratio was Wilis cultivar with 32.4%, and the lowest was Dering with 30.4%. Appressorium hyphae also cause cuticle and epidermis degradation, then hyphal colonies can form in the intracellular space and cause severe infection

(Braga et al., 2019). *P. pachyrhizi* infection in Slamet cultivar increased the palisade ratio by 70.2%, while in Wilis cultivar 46.5%, a pathogenic infection could cause hyperplasia of palisade tissue and change the shape of the cells from oval to isodiametric. In addition, pathogenic infection also causes hypertrophy of cells and causes tissue to be easily infected (Samiyarsih et

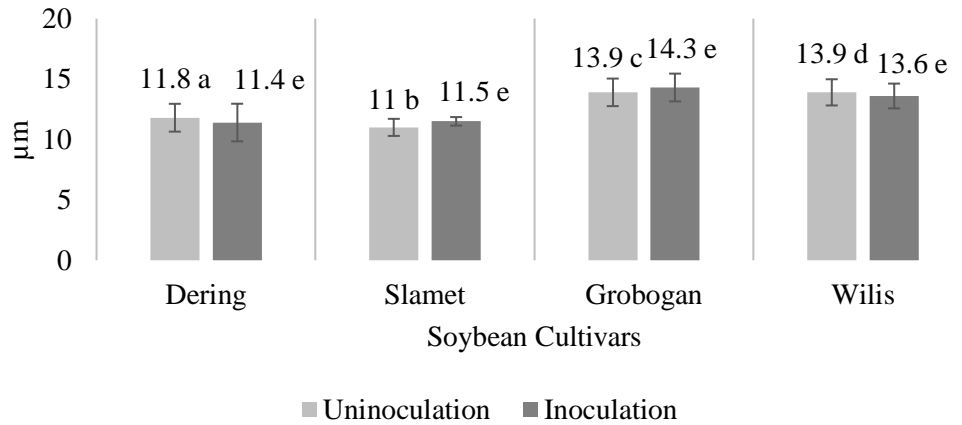


Figure 7. Stomata length of 4 soybean cultivars

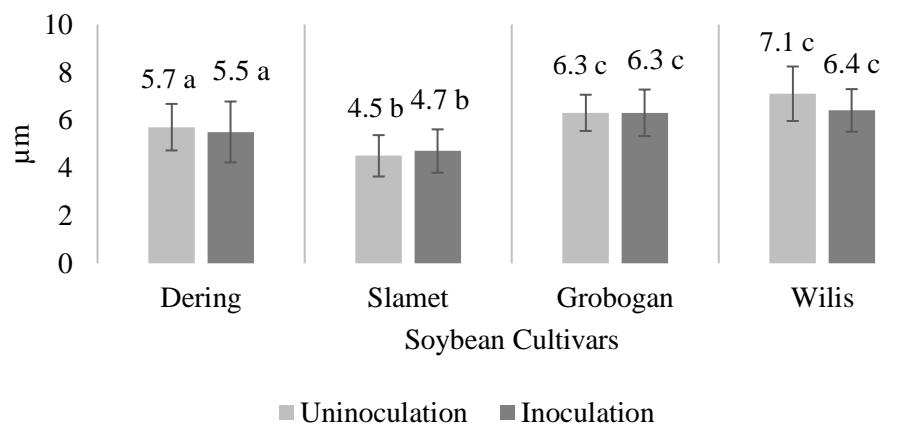


Figure 8. Stomata width of 4 soybean cultivars

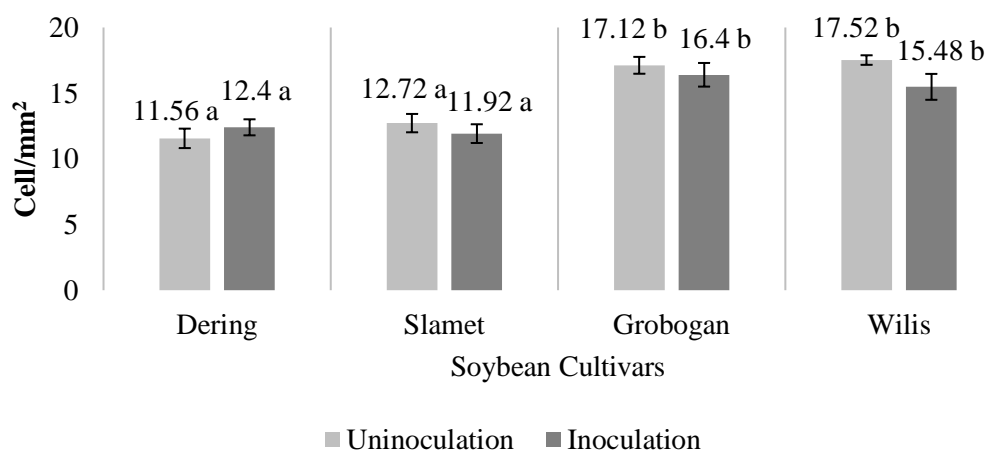


Figure 9. Stomata density (adaxial) 4 soybean cultivars

al., 2020).

Different results were shown in the measurement of stomata length of four soybean cultivars that were not inoculated and inoculated with *C. kikuchii*. The results of the LSD further test ($p < 0.05$) showed no significance. But overall, Dering and Slamet cultivars with the lowest

symptoms of blight infection (Figure 1) had the smallest stomata length compared to Grobogan and Wilis cultivars (Figure 7). Meanwhile, the results of the measurement of stomatal width in the four soybean cultivars that were not inoculated and inoculated with *C. kikuchii* also showed the same results, where the LSD further test was not

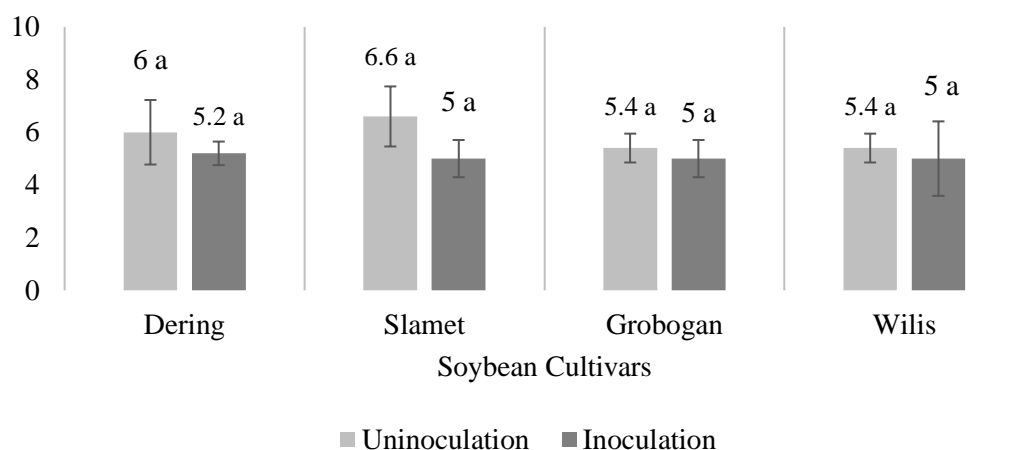


Figure 10. The number of trichomes of 4 soybean cultivars

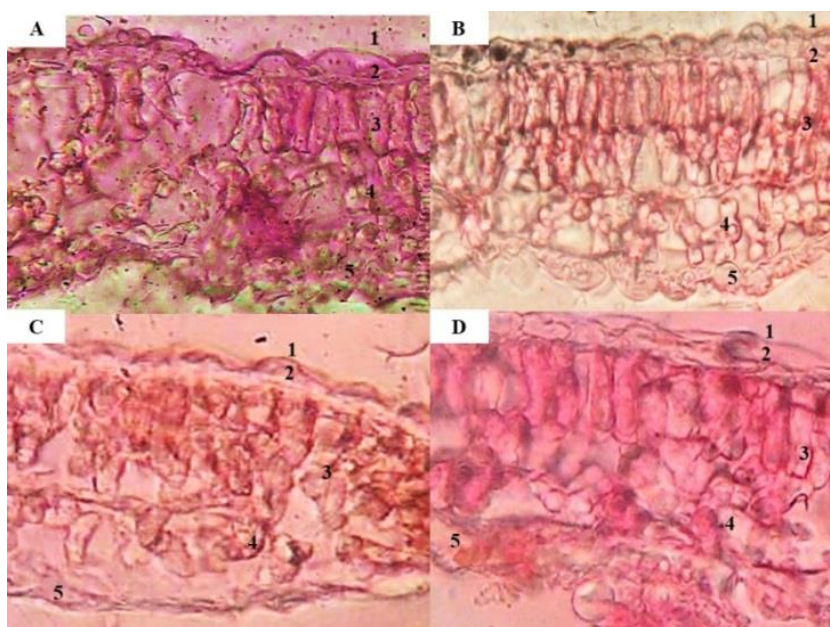


Figure 11. Anatomical leaf structure of 4 soybean cultivars after infection

Description: Anatomical Soybean Leaf Cultivar; Dering (A); Slamet (B); Grobogan (C); Wilis (D), after 21 days of *C. kikuchii* fungal inoculation. (1) Cuticle; (2) Epidermis (adaxial); (3) Palisade; (4) Sponge Tissue; (5) Epidermis (abaxial).

significant for each cultivar ($p < 0.05$) (Figure 8). Research has shown that stomatal pores play a role during pathogen attacks and under abiotic stress. Pathogens, pathogen-associated molecular patterns (PAMPs), and elicitors can be trigger stomatal closure (Fanourakis et al., 2020). Large stomatal openings provide a higher chance for pathogens to enter the tissue, and stomatal closure also depends on the perception of PAMPs (Sawinski et al., 2013).

Stomata are the main route for pathogens to penetrate. Stomata structure, frequency, and

distribution have been assumed to be significant variables affecting plant sensitivity and leaf resistance to pathogen entry (Dutton et al., 2014). The results of the measurement of stomatal density showed insignificant values in the LSD follow-up test ($p < 0.05$), but overall Dering and Slamet cultivars had stomatal density compared to the Grobogan and Wilis cultivars. The Dering cultivar that inoculated had a stomatal density of 12.4 cells/mm², while the Slamet cultivar was 11.92 cells/mm². The Grobogan that inoculated with *C. kikuchii* had a stomatal density of 16.4 cells/mm²,

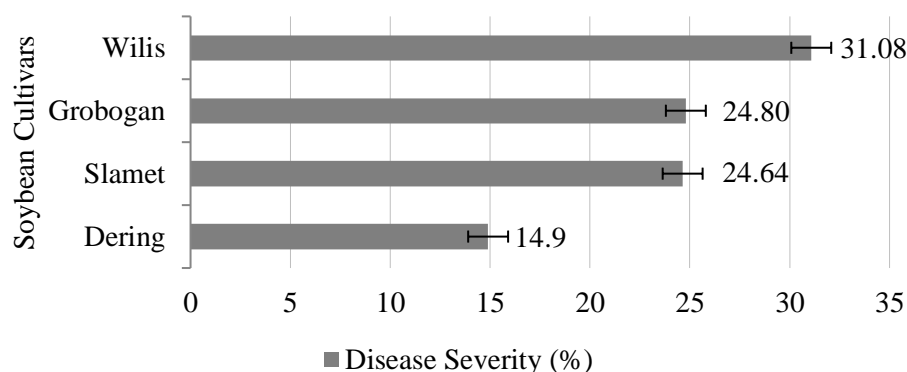


Figure 12. Disease severity of *Cercospora* leaf blight of 4 soybean cultivars

and the Wilis cultivar 15.48 cells/mm² (Figure 9).

These variations of stomatal density can be caused by differences in the environment in which they grow and the genetic factors that greatly affect stomatal morphogenesis. The number of stomata can also be influenced by adaptation to pathogen infection. Plant responses from pathogens can cause a decrease in stomatal density to prevent and adapt to minimize infection. Previous studies showed that the number of stomata on the adaxial and abaxial leaf taht infected with powdery mildew showed a significant correlation with the severity of the disease (Azmat et al., 2016). Low stomatal density can reduce the penetration and infection of

causing pathogens because trichomes can be a barrier for spores to germinate and minimize fungal spore deposition. Trichome can prevent pathogen infection in leaves by absorbing water and resisting infection (Irulappan & Senthil-Kumar, 2018).

Leaves with more trichomes are more resistant to pathogenic infections. This indicates that trichomes with all their characteristics (tight, long, and irregular structure) are the morphological resistance modalities against pests and disease-causing pathogens. Some experts report that soybean leaf trichomes have a close relationship with resistance to certain pests. The density of leaf trichomes inhibits the penetration of fungi,

Table 2. Categories and criteria for resistance of four soybean cultivars after 21 days of planting

Cultivars	Infected Leaves Positon	Average Number of Leaf Spot/Blight (cm ² of Leaf)	DS Value (%)	Score	Criteria	Infection Category (%)
Dering	3 rd Leaves	5	14.9	2.15	Resistant	DS < 25
Slamet	3 rd Leaves	14	24.64	2.85	Resistant	DS < 25
Grobogan	3 rd Leaves	15	24.80	2.92	Resistant	DS < 25
Wilis	3 rd Leaves	28	31.08	3.38	Moderate Resistant	DS > 25

pathogens (Samiyarsih et al., 2020).

The results of the measurement of the number of stomata showed an insignificant value in the LSD test ($p < 0.05$), but overall the number of stomata of Dering and Slamet cultivars in the uninoculated group had the highest number compared Grobogan and Wilis cultivars. Meanwhile, the inoculated group showed a relatively similar value, but when compared to the uninoculated group, it showed a decrease (Figure 10). A large number of trichomes in a plant can play a role in minimizing infection with disease-

inhibits hyphal growth, and minimizes spore germination (Putra et al., 2013). The number of trichomes can also be affected by a biotic and abiotic environment in which it grows (Pandey et al., 2017). Trichomes tends to increase in the dry season to suppress the rate of transpiration and decrease during the rainy season to facilitate the evaporation process (Aparecido et al., 2018). Histological observations of leaf tissue on four soybean cultivars inoculated with *C. kikuchii* showed different results (Figure 11).

Histological observations showed that the four

soybean cultivars inoculated with *C. kikuchii* showed changes in the histological structure with spongy tissue sections showing damage, especially in the Grobogan (C) and Wilis (D) cultivars. While the palisade tissue structure was very compact and tight, especially seen in Slamet cultivar (B), the epidermis (abaxial) appeared to show severe damage in Grobogan and Wilis cultivars. The results showed that Dering cultivar had the lowest disease severity with 14.9%. Slamet cultivar has almost the same disease severity as Grobogan cultivar, where Slamet cultivar is 24.64% and Grobogan cultivar is 24.80%. Meanwhile, the Wilis cultivar had the highest disease severity level, which was 31.08%. Based on the data obtained from the percentage value of disease severity, Dering, Slamet, and Grobogan cultivars were classified into the criteria for plants that were resistant (R) of *C. kikuchii* fungal infection (Figure 12).

The percentage of DS Dering, Slamet, and Grobogan scores is less than 25%. Different things were shown in the Wilis cultivar which had the highest value (>25%) and was classified as a soybean plant that was somewhat moderate resistant (MR) of *C. kikuchii* infection (Ngegba et al., 2017). The existence of a variety and difference as well as the susceptibility of a plant to certain pathogens can be caused by differences in the genes controlling the resistance of a plant, the level of pathogenicity, and environmental factors that influence these genes. Resistance genes also vary from small to large, because it depends on the function controlled by the gene itself, include the virulence gene such as Race15 and Race1 of *C. sojina* in causing disease (Gu et al., 2020).

The existence of a morphological difference between cultivars is thought to be one of the factors of resistance to a disease-causing pathogen. Soybean plants with moderate disease resistance have stiffer or thicker leaves with darker colors (Jamir et al., 2020). Plants with increased resistance often have xeromorphic structures such as smaller and thicker leaves, more epidermal trichomes, smaller and denser stomata, thicker cuticle epidermis, and thicker palisade tissue (Fang & Xiong, 2015). Aliyah et al. (2015), stated that the leaves of resistant cultivars have a dark color, stiff and strong, and thick epidermis.

The Dering cultivar category which is resistant to *C. kikuchii* infection is greater than Slamet, Grobogan, and Wilis. The results of observations of *Cercospora* leaf blight symptoms began to appear on the 7-11th day after inoculation with an average on day 10 and the length of observation

starting from day 0 to day 2. The response shown by each cultivar was different, especially in the Dering and Slamet cultivars, where the initial symptoms appeared longer compared to Grobogan and Wilis cultivars, although the average incubation period was the same. The results also show that the average temperature in the morning is 27°C, during the day 23.39°C, and at night 30.77°C. while the humidity in the morning is 60.87%, during the day 42.35%, and at night 51.60%.

High humidity >60% and a temperature of 22.7-26.7°C are very suitable for the development of the pathogen *C. kikuchii*. The initial infection is often latent (asymptomatic). Pathogens that infect the pods and can grow will penetrate the seed coat (Inayati & Yusnawan, 2017). Continuous high humidity in stable conditions for the inoculum can shorten the period required for *C. kikuchii* disease progression (Kashiwa et al., 2021). The initial stage of *C. kikuchii* infection occurs when the spores attach to the leaf surface. *C. kikuchii* hyphae can penetrate directly through epidermal cells as well as through stomata on leaves (Andrade et al., 2021). *C. kikuchii* infection produces a red light-activated pigment perylene quinone called cercosporin that diseased tissue to turn purplish in color and is toxic to plant cells (Li et al., 2019).

The results of the study on epidermal thickness, cuticle, mesophyll, and palisade ratio showed an interaction between four soybean cultivars with *C. kikuchii* inoculation treatment. Response of plants to various pathogenic infections can produce morphological, anatomical, and physiological variations, such as changes in cell structure, decreased leaf area, increased leaf thickness and stomata sensitivity (Pandey et al., 2017).

CONCLUSION

Dering and Slamet cultivars had the highest cuticle thickness, epidermis, and palisade ratio and the lowest stomata length and width with the highest number of stomata and trichomes, compared to Grobogan and Wilis cultivars. The resistance level of Dering, Slamet, and Grobogan cultivars against cercospora leaf blight caused by *C. kikuchii* was classified into a resistant cultivar with a DS value <25%, and a Wilis cultivar was classified into a moderately resistant (MR) category, with a DS value of 31.08%. Cultivar selection against CLB disease is important and its effectiveness for increasing soybean productivity based on anatomical characteristics and resistance.

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