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Downy Mildew Infection in Indonesian Melon Cultivar 'Melona' Based on Morphological and Anatomical Characters

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Abstract

Melons are a horticultural crop of the Cucurbitaceae family with high economic value and worldwide distribution. The 'Melona' variety is the result of breeding selection from commercial melons in Indonesia and has a golden yellow skin color with lobes, crisp flesh, and a high degree of sweetness. Downy mildew is a fungal infection that attacks the leaves of the plant causing brownish-yellow-colored symptoms. Downy mildew can cause crop failure under extreme conditions as the plant loses its productive capacity. Infection levels were observed by scoring and calculating disease index scores. The morphology of healthy and diseased plants was documented and analyzed descriptively. Anatomical features of healthy and diseased leaves were compared using anatomical observations on leaf cross-sections. Quantitative data analysis was carried out using the T-test analysis method with a 5% significance level. Melona's resistance to downy mildew is in the tolerant to susceptible category. Chlorosis and necrotic spots on the leaves and stunted growth are symptoms of downy mildew infection. Healthy leaves had a greater axial and abaxial epidermal thickness, a lower mesophyll thickness, a smaller cell size and a better quantity and quality of trichomes than leaves infected with downy mildew.

Keywords: Downy mildew disease, fungal infection,plant response, resistance level

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Introduction

The Cucumis genus is most widely cultivated member of the Cucurbitaceae family because of its high economic value (Daryono & Maryanto, 2018). Various melon cultivars; with its own unique character; have been produced in plant breeding. 'Melona' is the result of selection breeding of the commercial cultivar (Yusuf et al., 2023). Melon 'Melona' has a golden yellow or orange skin color when ripe, lobulated fruit shape, and sweet-tasting fruit flesh. Each 'Melona' fruit is medium in size and weighs about 1.5 kg. The flesh of the 'Melona' fruit is orange with a crunchy texture and the level of sugar content (brix) ranges from 14 - 16. The planting time for 'Melona' cultivar is 65 - 80 DAP until it is ready for harvest (Alfiani, 2017; Latifah, 2016). Melon consumption remains high for several reasons, including their nutritional value, delicious taste, and wide cultivation, which helps maintain stable prices across different economic classes (Torres *et al*., 2020).

One of the greatest obstacles in plant cultivation is the decline in crop productivity and yields due to plant diseases (Ojiambo *et al*., 2015). Downy mildew, a fungal disease caused by airborne oomycete pathogens of *Pseudoperonospora cubensis*, causes destructive symptoms in melon plants (Sun *et al*., 2022; Thines, 2014). This infection has led to significant losses worldwide in crops such as melons, cucumbers, watermelons, and squash (Holmes *et al*., 2015). Bacterial antagonists to *P. cubensis* have been developed to reduce the need for pesticides, which negatively impact environmental quality (Zheng *et al*., 2018).

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Inter-simple sequence repeat (ISSR) markers show promise in the early screening of resistant and susceptible cucumber varieties (Innark *et al*., 2014), providing a faster alternative for identifying excellent downy mildew-resistant lines compared to the complexity of traditional breeding methods for creating disease-resistant plants (Holdsworth *et al*., 2014).

The symptom of downy mildew is the appearance of yellowish chlorotic lesions that are localized and generally have an irregular shape. On melon and watermelon plants, the lesions are generally not limited by leaf veins and tend to be circular or irregular in shape (Wen *et al*., 2019). Infection with very high severity is characterized by necrosis of the leaves that can be followed by the death of infected plants (Lebeda *et al*., 2016). The severity of downy mildew infection and the response of plants in each variety can vary depending on the level of susceptibility and sensitivity. At high levels of severity, decreased metabolite production due to reduced photosynthetic active area can causing a significant reduction in crop yields (Call *et al*., 2013).

Plant resistance to pathogens is determined by the strength and effectiveness of the defense system. Passive defense includes the anatomy and morphology of plants that provide structural defense. Meanwhile, active defense is present in the form of a hypersensitive response, the synthesis of compounds that are toxic to pathogens, or the activity of reactive oxygen species (Pradana *et al*., 2017). By comparing the morphological and anatomical characters of healthy and infected plants, this study aims to analyze the resistance of 'Melona' cultivar to downy mildew.

Methods

Research Area

This research was conducted from May to August 2023. The research consisted of three major parts: plant growing and maintenance, sample collection and data observations of morphological and anatomical characteristics. Plant cultivation and maintenance were carried out in the open field and screenhouse at Mutihan, Madurejo, Sleman, Yogyakarta. Anatomical observations were carried out at the Plant Structure and Development Laboratory, Faculty of Biology, Universitas Gadjah Mada.

Melon cultivation

Land preparation for conventional cultivation includes ploughing to loosen the soil, fertilization, making beds, and irrigation. Seeds are sown in tray pots that have been sterilized with insecticides and fungicides. Sprouts that have grown are transferred to polybags with planting media at the age of seven days. For approximately 3 months, Melona plants were maintained to optimize growth with insecticide application and regular irrigation. Fungicide treatment was not applied to determine the response of Melona plants to downy mildew fungal infection.

Morphological observation and scoring of infection

Morphological observations were performed by identifying the presence and absence of clinical symptoms that indicate downy mildew infection. Symptoms observed included leaf characteristics such as leaf color appearance and the presence of yellow chlorotic spots. Scoring of downy mildew infection rate in 'Melona' melon population was carried out at the age of 40, 45, and 50 days after planting (DAP). The percentages calculated in scoring the infection rate include the percentage of leaf, plant, and population infection rates. The formula used in calculating the scoring follows the research of (Ishak & Daryono, 2020).

$$\text{Leaf Infection} = \frac{\text{Number of boxes infected}}{\text{Total number of leaf boxes}} \times 100\ \%$$

$$\text{Plant Infection} = \frac{\text{Sum of leaf infections}}{\text{Number of leaves in the plant}} \times 100\ \%$$

$$\text{Population Infection} = \frac{\text{Sum of Plant infections}}{\text{Number of plants in the population}} \times 100\ \%$$

The results of scoring the percentage of infection on the leaves were converted into an ordinal disease index (DI) score consisting of six levels based on the area of symptomatic leaves, as follows in the Table 1. Resistance of a plant based on disease index (DI) score can be divided into three categories: DI score 0-1 as resistant group, DI score 2-3 as moderately resistant group, and DI score 4-5 as susceptible group. The infection rate in the plant population in the resistant category ranges from 0-10%, the tolerant category ranges from 11-50%, while the susceptible category ranges from 51-100% (Fukino *et al*., 2004).

Table 1. Disease Index assessment of infection symtomps

Disease Index Score	Infection Symptoms
1	No symptoms
2	1% - 10% leaf area symptomatic
3	11% - 30% symptomatic leaf area
4	31% - 50% symptomatic leaf area
5	51% - 80% symptomatic leaf area
6	81% - 100% symptomatic leaf area

Anatomical observation of leaf samples

Anatomical observations were elucidated in several day activities. In the first time, the samples were fixed using FAA solution consisting of glacial acetic acid, formalin, and alcohol in the ratio of 5 : 5 : 90. The treatment continued after 24 hours with gradual removal of the fixative solution using 70% alcohol, 80% alcohol, 95% alcohol, and 100% alcohol for 30 minutes each rinse. Dealcoholizing was carried out using alcohol: xylol 3:1, alcohol: xylol 1:1, alcohol: xylol 1:3, xylol, and xylol each for 30 minutes. Next, xylol was discarded and replaced with a mixture of xylol: paraffin 1:9 with a temperature of 57^{o}C for 24 hours.

The next 24 hours was infiltration. Infiltration was performed using pure paraffin at 57^{o}C for 24 hours. On the next day, paraffin was replaced with new pure paraffin into the petridish containing the sample, after one hour the box containing the sample was ready for observation. The samples were then sliced with a rotatory microtome with a thickness of 12-15 mm. The slices were attached to a glass slide with a mixture of glycerin: albumin spiked with water. The glasses were placed on a hot plate with a temperature of 45^{o}C until the paraffin ribbon was stretched.

Staining using 1% safranin in 70% alcohol. The glass objects were placed in a solution of xylol, xylol, alcohol: xylol 1:3, alcohol: xylol 1:1, alcohol: xylol 3:1, alcohol 100%, alcohol 100%, alcohol 95%, alcohol 80%, alcohol 70%, each treatment was carried out for 3 minutes. Samples were then placed in 1% safranin in 70% alcohol for 1 hour, followed by 70% alcohol, 80% alcohol, 95% alcohol, 100% alcohol, 100% alcohol, 3:1 alcohol: xylol, 1:1 alcohol: xylol, 1:3 alcohol: xylol, xylol, and xylol sequentially for 1 minute for each treatment. The preparations were then covered with Canada balsam and dried on a hot plate with a temperature of 45^{o}C until the balsam dried. The preparations were then observed with a microscope connected to optilab.

Data Analysis

Qualitative data were analyzed descriptively. Quantitative data were analyzed using the Independent Sample T-test and Mann Whitney U-test. The T-test is a statistical test that aims to compare the means of two mutually independent sample groups to determine whether there is a statistically significant difference between the two. Meanwhile, the Mann Whitney U-test analyzes the median of two independent groups as a non-parametric test when the dependent variable data scale is ordinal or invertal or ratio which is not normally distributed.

Results and Discussion

Downy mildew symptoms based on morphological characteristics.

Leaf morphology of healthy plants and plants infected with downy mildew is shown in Figure 1. Symptoms of the disease observed on the appearance of melon leaves infected with downy mildew were chlorosis of the leaves with a yellowish color which gradually spread and turned into necrosis of brownish leaves. In addition, plant growth is not optimal and some experience stunted growth. The colors of 'Melona' healthy leaves based on the Royal Horticultural Society (RHS) Color Chart is moderate olive green (RHS 137B) on the adaxial surface and moderate yellowish green (RHS 138B) on the abaxial surface. The basic color of melon leaves infected with downy mildew is moderate yellowish green (RHS 146C) on the adaxial surface and strong greenish yellow (RHS 151B) on the abaxial surface. In the early phase, infection of mild severity is characterized by the appearance of primary light-yellow lesions (RHS 17D).

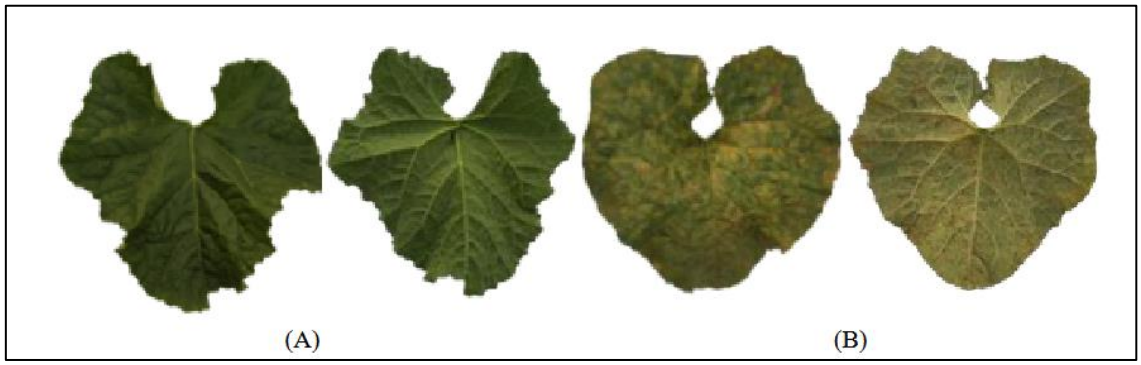

Figure 1. Adaxial and abaxial appearance of the 'Melona' leaves. (A) healthy and (B) infected by downy mildew.

Progression of disease symptoms to infection of moderate severity is indicated by the joining of the primary lesions to form larger lesions. In general, at this stage the spots appear clearer with a striking yellow color, namely brilliant yellow (RHS 15C) or vivid yellow (RHS 17B). Infection with a high level of severity is characterized by changes in areas of chlorosis to necrosis so that the leaves are brownish orange (RHS 166C) to moderate brown (RHS 165A). Symptoms of downy mildew are characterized by irregular features that are not limited by leaf veins and typically do not exhibit pathogen sporulation (Crandall *et al*., 2018; Holmes *et al*., 2015).

Downy mildew infection rate

The downy mildew infection rate in the population of 'Melona' are presented in Table 2. Based on the Table 2, The value of the infection rate in the 'Melona' population at the age of 45 days after planting (DAP) to 50 days after planting ranged from 24.56 - 52.59%. 'Melona' is categorized as a tolerant plant at 40 DAP, the resistance category 'Melona' changes to susceptible at 50 DAP. The infection rate continues to increase significantly as the plant ages. Yusuf *et al*., (2023) stated that the average age of melon plants ranges from 55-65 DAP. In this observation the plant has experienced a stationary period or experienced senescence. Stationary period is a condition characterized by the absence of division, while senescence is a condition of increasing plant age accompanied by a decrease in conditions. These events reduce plant resistance and lead to cell damage and death (Taiz *et al*., 2015)

Anatomical observation of leaf

The anatomical structure of healthy melon leaves 'Melona' and 'GMP' from top to bottom consists of upper epidermis, palisade mesophyll, spongy mesophyll, and lower epidermis tissue, respectively as shown in Figure 2. In healthy plant leaves, the epidermal and palisade mesophyll tissues have cells that are tightly arranged and regular. Air space between cells is small or almost non-existent. Epidermal derivatives in the form of trichomes that appear intact are found in the upper and lower epidermis. In contrast to the anatomical structure of healthy leaves, trichomes are rarely found on the upper or lower epidermis of infected leaves, if they are present, they are generally incomplete or damaged. In infected leaves, the cells that make up the epidermal and palisade mesophyll tissues are larger in size but are arranged in a less dense and irregular manner. The air space between the sidelines of the cell looks bigger.

Table 2. Downy mildew infection rates in the population of 'Melona' cultivar

Days after planting (DAP)	Infection rate (%)	Category
40	24,56%	Tolerant
45	34,72%	Tolerant
50	52,59%	Susceptible

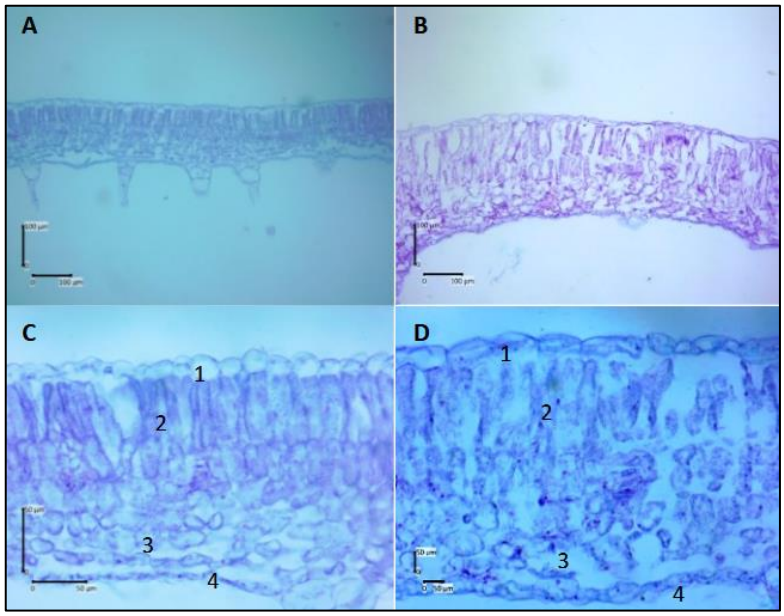

Figure 2. Anatomy of the healthy (A-C) and infected by downy mildew (B-D) 'Melona' leaves. (1) upper epidermis, (2) palisade mesophyll, (3) spongy mesophyll, (4) lower epidermis, (5) trichome. Magnification: A-B 10x; C-D 40x

Glandular and non-glandular trichomes have different functions in plant defense mechanisms. The presence of non-glandular trichomes as epidermal derivatives facilitates plants in regulating surface temperature, preventing excess water loss, reducing excess moisture or wetting, and helping to absorb water and nutrients. Meanwhile, the presence of glands in the glandular trichomes allows for additional functions which is secreting certain compounds that are toxic to pathogenic organisms, thus becoming a defense structure in preventing pests and diseases. Compounds secreted by glandular trichomes accumulate on the leaf surface and provide initial protection against plant-infecting pathogens through direct contact (Schuurink & Tissier, 2020). Trichomes in the epidermal tissue of melon leaves may reduce leaf moisture, resulting in inhibition of the development of downy mildew. This is because water or leaf moisture is an essential factor in the early stages of *P. cubensis* infection to initiate germination and form primary infectious structures in leaves. Conditions of low leaf humidity will inhibit the germination of sporangia of *P. cubensis* (Holmes *et al*., 2015).

Quantitative analysis of leaf anatomy

The result of quantitative analysis of the leaf anatomical characters is shown at Table 3. Structural defense in plants is an important factor in resistance to pathogen infection. Leaf anatomy: for example the leaf epidermis can vary in response to adjustments to environmental factors and biotic stress (Ni *et al*., 2022). Anatomical structures such as the thickness and quality of the cuticle, the structure of the epidermal cell wall, and thick epidermal tissue can provide a passive resistance mechanism for plants to inhibit pathogenic infections (Serrano *et al*., 2014). The cuticle and epidermis are the main barrier layers that function to protect the tissue from the external environment by limiting the intercellular space (Pradana *et al*., 2017). The level of protection of the epidermis as the main tissue that plays a role in structural defense is determined by the characters in the form of size, number, thickness, cell wall structure, and the condition of the cuticle layer in the epidermal tissue. The thick epidermis and cuticle layer have a higher possibility of providing resistance to pathogenic fungal infections that penetrate directly (Serrano *et al*., 2014). The thickness of the cuticle generally supports the thickness of the epidermis. The thicker and stronger the outer wall of the epidermal cells will effectively inhibit or inhibit the penetration of pathogenic fungi (Dewi *et al*., 2013).

Table 3. Quantitative analysis of the anatomical characters

Anatomical Parameters	n	Mean ± SD	Mean Differences	Sig.
Adaxial Epidermis Thickness				
Healty plant	25	20,23±2,58 µm	11,71	0
Infected plant	25	8,51±1,04 µm	(10,51-12,91)	
Adaxial Epidermal Cell Length				
Healty plant	25	20,64±4,16 µm	7,24	0
Infected plant	25	27,88±5,53 µm	(4,40-10,07)	
Palisade Mesophyll Thickness				
Healty plant	25	68,90±17,77 µm	14,98	0
Infected plant	25	83,89±8,74 µm	(7,91-22,06)	
Palisade Mesophyll Cell Width				
Healty plant	25	12,81±1,67 µm	18,47	0
Infected plant	25	31,29±4,77 µm	(16,41-20,53)	
Spongy Mesophyll Thickness				
Healty plant	25	67,63±16,15 µm	36,35	0
Infected plant	25	102,51±26,50 µm	(28,93-43,76)	
Abaxial Epidermal Thickness				
Healty plant	25	15,74±2,51 µm	6,77	0
Infected plant	25	8,97±0,72 µm	(5,66-7,88)	
Abaxial Epidermal Cell Length				
Healty plant	25	18,27±2,78 µm	5,92	0
Infected plant	25	24,20±3,85 µm	(3,61-8,23)	
Leaf Thickness				
Healty plant	25	165,98±26,53 µm	37.98	0
Infected plant	25	203,96±25,10 µm	(23,41-52,55)	

The difference in thickness of palisade and spongy mesophyll between healthy leaves and infected downy mildew is caused by changes in histological forms, namely hyperplasia and hypertrophy in the tissue (Biruliova *et al*., 2013). Hyperplasia is a condition when there is excessive proliferation of tissues so that the number of cells increases, while hypertrophy is an increase in cell size. Faster cell division occurs in leaves infected with pathogens so that the cells change size (Pradana *et al.*, 2017). Pathogenic infections can cause thickening of the mesophyll tissue due to tissue swelling (Samiyarsih *et al*., 2018, 2022). This event may also be influenced by the excess auxin hormone synthesized by plants infected with pathogens. Mesophyll cells will also experience deformation and lose chlorophyll gradually. Deformation causes the shape of the cell to appear unclear or irregular. In the event of downy mildew infection, this is probably caused by the release of enzyme substances that function to support cell penetration and development of hyphae in the mesophyll tissue.

Pathogens can temporarily or permanently promote water loss by increasing surface evaporation along the development of infectious structures on the surface of plant organs, i.e. by damaging the cuticle of tissues and causing cell death leading to uncontrolled loss of water (Wang *et al*., 2015). Post-infection through stomatal penetration, *P. cubensis* grows in the intercellular space and forms haustoria through invagination of the plasma membrane in living cells. This process is carried out without producing toxins, but the production of enzymes in certain amounts that function to degrade extracellular cell walls (Kubicek *et al*., 2014).

In resistant host plants, the failure of P. *cubensis* to penetrate mesophyll cells can also be caused by the accumulation of callose and lignin along the cell wall and the inner surface of the mycelia wall. This response is followed by the accumulation of dense dark colored material in the cytoplasm of the host cell which leads to necrosis. Changes in metabolic activity in the form of phenolic accumulation and increased peroxidase activity are also growth inhibitors for pathogen (Ferreira *et al*., 2013).

Conclusions

The melon cultivar 'Melona' has resistance to downy mildew at a tolerant level

up to 45 days after planting. At the age of 50 days after plantation, the resistance level of 'Melona' decreased to a susceptible level. The morphology of leaves infected with downy mildew showed symptoms of infection in the form of yellowish chlorosis spots and brownish areas of necrosis of various sizes which were not found on leaves of healthy plants. Leaves of healthy plants had thicker adaxial and abaxial epidermal tissue anatomical characteristics, thinner mesophyll tissue, smaller mesophyll cell size, and better quantity and quality of trichomes compared to leaves infected with downy mildew.

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