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Tolerance of Melon (*Cucumis melo* L.) Cultivar Melodi Gama 3 to *Powdery Mildew* Infection

Rozikin[1,2], Budi Setiadi Daryono[1](*)
[1]Fakultas Biologi Universitas Gadjah Mada Jl. Teknika Selatan, Sleman, D.I. Yogyakarta, Indonesia. 55281;
[2]Fakultas Kedokteran, Universitas Islam Al-Azhar, Jl. Unizar No.20, Turida, Sandubaya, Mataram, Nusa Tenggara Barat, 83232, Indonesia

***Corresponding author**: bs_daryono@mail.ugm.ac.id

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Abstract

Powdery Mildew is one of the most important melon diseases caused by ascomycetes fungi belonging to the genus of Sphaerotheca and Erysiphe. Breeding is considered the best approach to control Powdery Mildew infection in melon. MG3 is a melon cultivar derived from 'MG1' x 'Ladika' and has been estimated to carry a resistant gene on Powdery Mildew infection inherited from MG1. The aim of this research is to find out the percentage and phenotype characters of Powdery Mildew infection in MG3 compared to 4 commercial melons, such as Action 434, Glamour, MAI and Ladika that are popular in the market. Five melons are cultivated in greenhouse B of KP4 UGM. After 5 weeks, the plants were inoculated with Powdery Mildew in the leaves area. The percentage of disease infection was measured after 3 days inoculation for 8 times in 24 days with a grid line (21 cm x 21 cm with small cubics 1,5 cm x 1,5 cm). The analysis of infection level was done using Fukino protocol. The results showed that cultivar MG3 was tolerant and stable to Powdery Mildew infection compared to cultivar Action 434, MAI and Ladika in leaves, plant and population level. The infection of Powdery Mildew caused severe leaf distortion and decreased the ability to photosynthesize in leaf tissue resulting in necrosis, chlorosis and death to the infected leaves. In fruit, infection causes break in the net, immature and rotten flesh, decrease in sweetness and scent in cultivar Action434, MAI and Ladika. According to microscopic analysis, isolate KP4 UGM belongs to a member of Podosphaera.

Keywords : *Breeding, Melon, MG3, Powdery Mildew*

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INTRODUCTION

Melon (*Cucumis melo* L.) is a horticultural crop that has been grown in Indonesia since 1980. Melon belongs to the Spermatophyta division and the Angiospermae subdivision because it sexually reproduces itself through its flower and produces seeds which characteristics are covered or protected by sporophylls, transport bundles on the stem having trachea, microsporangia found in microsporophylls (stamens), and flower jewelry.

As a member of the Dicotyledonae class and the Sympetalous subclass, melons have wet stems, flower corolla in multiples of four or five, flower corolla attached to each other, and a taproot and embryo with two cotyledons. Melon is part of the Cucurbitales order and the Cucurbitaceae family because it has convoluted tendrils that are metamorphoses of branches, branches, or supporting leaves. *Powdery Mildew* is one of the diseases that affect melon plants. Ascomycetes of the genera Uncinula, Phyllactinia, Podosphaera, Microsphaera, Sphaerotheca, and Erysiphe are responsible for this disease. Pumpkin plants (Cucurbitaceae), particularly melons, are specific hosts for Sphaerotheca and Erysiphe.(Cao et al., 2021; Cui et al., 2022; Ivanova et al., 2018; Wang et al., 2021).

Farmers use chemical fungicides that are not environmentally friendly and increase resistance to fungi, rendering them ineffective. Aside from fungicides, various attempts have been made to treat the *Powdery Mildew* infection, one of which is cultigen. Due to its inherited resistance from the parents, this approach is effective in controlling *Powdery Mildew*. Breeding of melon cultigen is done by crossing melons that are resistant to *Powdery Mildew* with non-resistant melons to study and determine resistance genes inherited in populations. For melons with female parent PI 371795, resistance against *Powdery Mildew* is determined by a single dominant gene (pm-I) (H. Cui et al., 2022; L. Cui et al., 2022).

Study on *Powdery Mildew infection* has been progressing since the 1980s until today. Researchers start using genetic and molecular approaches to further study the genes influencing and controlling resistance to *Powdery Mildew* infection in important horticultural crops including melons to find the best solution to control them. (Daryono et al., 2011). Genetic sequencing analysis in melon plants and *Powdery Mildew* provide better understanding of the position of chromosomes and genes that influence resistance in *Powdery Mildew* (Garcia-Mas et al., 2012; Natarajan et al., 2016).

Melodi Gama 3 (MG3) melon cultivar is a crossbreed between Melody Gama 1 and Ladika cultivars, which brings the superior properties of the female parent (MG1), among them is resistance to *Kyuri Green Mottle* Mosaic Virus (KGMMV-YM) Indonesian isolates and *Cucumber Mosaic Virus* CMV-B2) Indonesian isolates (as well as resistance genes to *Powdery Mildew)*. MG3 itself has been studied to have tolerance to KGMMV infection (Daryono & Fitriyah, 2016).

METHOD

In this study, a quantitative approach was used to score the level of infection in both individual plants and populations using the method of Fukino *et al* (2004). The calculation is performed using the formula below:

$$\% \text{ Rate of leaf infection} = \frac{The\ number\ of\ infected\ leaves\ on\ a\ single\ leaf}{The\ total\ number\ of\ leaf\ boxes}\ x\ 100\%$$

$$\% \text{ Rate of infection in one plant} = \frac{Each\ leaf's\ total\ number\ of\ infections}{number\ of\ leaves\ on\ a\ single\ plant}\ x\ 100\%$$

$$\% \text{ Rate of infection in one population} = \frac{The\ amount\ of\ infections\ per\ plant}{number\ of\ plants\ within\ 1\ population} x\ 100\%$$

Tools and Materials

The following materials and tools were used for this research: six melon seeds from each cultivar of MG3, Action 434, Glamour, Mai, and Ladika, Action 434 melon leaves that have been infected with *Powdery Mildew (Podosphaera Xanthii)* isolates KP4 UGM, soil sample from Sleman, compost, vermicompost fertilizer, tap water, papers and NPK, ZA, Urea and TSP (SP-36) fertilizers, rulers; scales, *grid line* size 10 cm x 10 cm and 20 cm x 20 cm with small plots measuring 0.5 cm x 0.5 cm and light microscope.

Table 1. Resistance test index for melon plants against *Powdery Mildew*

Disease index score	Symptom
0	No symptom
1	1% - 10% leaf area affected
2	11% - 30% leaf area affected
3	31% - 50% leaf area affected
4	51 % - 80% leaf area affected
5	81% to 100% leaf area affected

Procedures

MG3, Action 434, Glamour, Mai, and Ladika melon seeds are planted in pots and then incubated in the *greenhouse* B KP4 UGM. The *Powdery Mildew* was inoculated after the plants had reached 4 weeks old with around 10–15 leaves. Each leaf of the examined plants was inoculated with the pathogen through direct physical contact with infected leaf.

Disease infection of *Powdery Mildew* was determined after three days of inoculation using grid line scoring procedure with dimension at 10 cm x 10 cm and 20 cm x 20 cm and also with narrow spaces at 0.5 cm x 0.5 cm. The calculated rates have included the percentage of leaf infection rates, the infection rate in plants, and the population infection rate of the *Powdery Mildew* fungus according to Fukino et al., (2004). The changes of morphological shape and color of the leaves and fruits and organoleptic in the fruit's flavor were also calculated among the morphological characteristics of the plants sampled. Furthermore, characteristics of *Powdery Mildew* were also identified under a light microscope to determine the key factor for identification on the genus or species level.

RESULTS AND DISCUSSION

Tolerance of MG3 Melon vs. Commercial Melon to *Mildew Powder Infection*

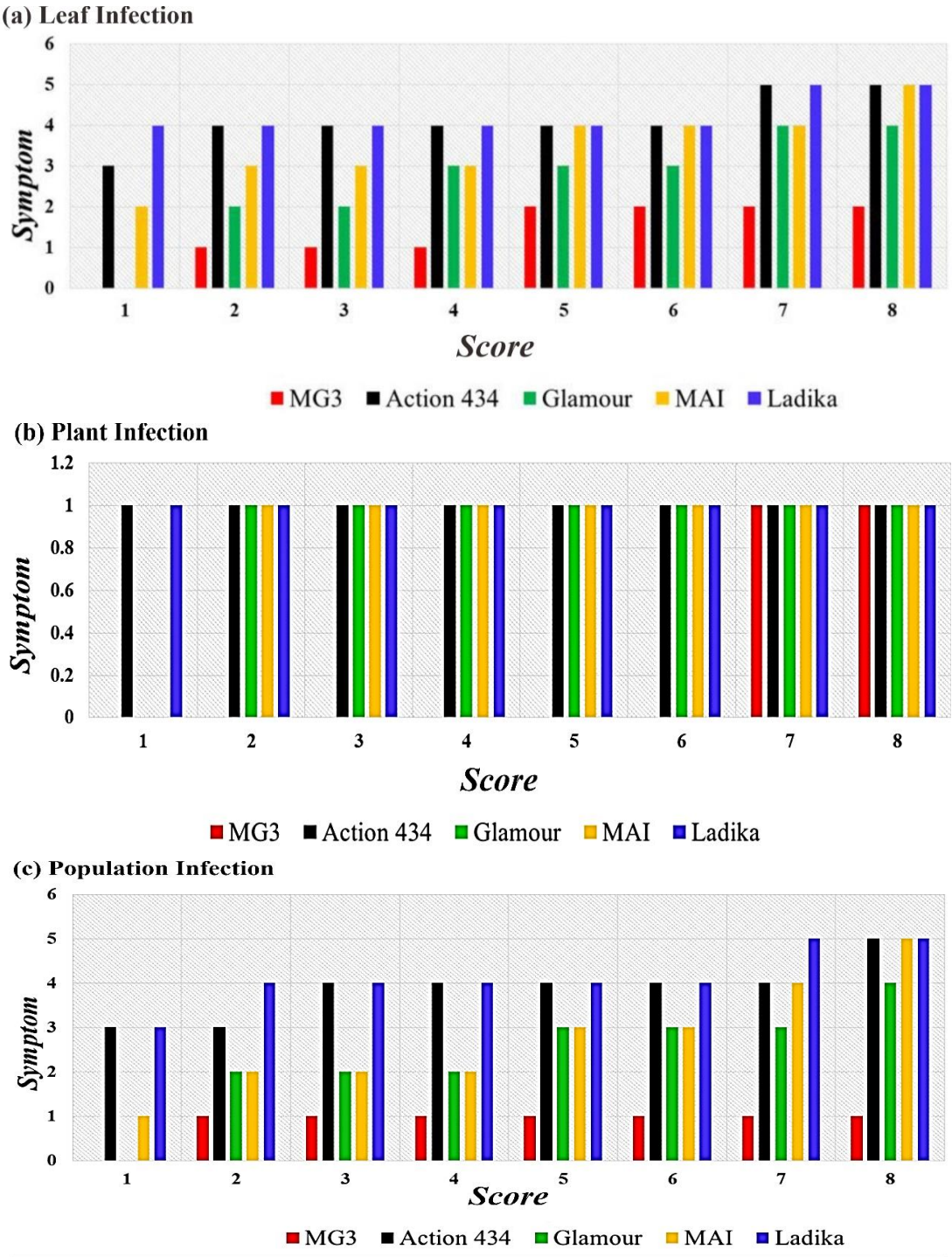

Figure 1. *Powdery Mildew* infection at the level of leaves, plants and populations (a-c) in the MG3 cultivar melon (Red graph), Action 434 (Black graph), Glamour (Green graph), MAI (Yellow graph) and Ladika (Blue graph) with an infection percentage scale of 1 (1%-10%); 2 (11%-30%); 3 (31%-50%); 4 (51%-80%); 5 (81%-100%).

In *greenhouse* B KP4 UGM, the proportion and characteristic of MG3 resistance on *Powdery Mildew* infection were evaluated. Pathogen was inoculated to the melon cultivar MG3 and also to the other four commercial melons. The inoculum was taken from infected leaves aseptically to the *greenhouse* A KP4 UGM, and then inoculated by direct contact to the healthy leaves of cultivar Action 434 melon. This cultivar was chosen as a host for the multiplication of KP4 isolate, due to its susceptibility to fungal infections. The inoculation was done by direct contact from infected leaf to the healthy leaf from each the remaining cultivars.

The percentage of infected cells in the leaves of melon cultivar MG3 ranged from 0.044% to 19,87% (Fig. 1A). In the 1st until 8th scoring, Cultivar Action 434, Glamor, MAI, and Ladika showed significant levels of infection, by the following: 49.55% – 94.75%, 0.11% – 70.22%, and 11.25% – 100%. The results indicated that cultivar MG3 has a higher resistance against *Powdery Mildew* compared to other cultivars. Disease infection level was lower in melon cultivar MG3 compared to the other four commercial melons (Fig. 1B), by the following percentage: 0.003% – 1.32% in MG3; 2.08% – 3.98% in Action 434; 0.004% – 2.76% in Glamor; 0.42% – 3.00% in MAI; and 2.75% – 4.71% in Ladika. Meanwhile, at the population level (Figure 1C), cultivar MG3 showed insignificant increase in the infection rate from the first to the last observation, 0.024% – 19.87%. In contrast, the other remaining cultivars significantly increased in the infection rate, by the following percentage: 49.55% - 94.75% in Action 434; 0.114% - 70.22% in Glamor; 11.25% - 81.49% in MAI; 58.41% - 100% in Ladika. These results indicated that the cultivar MG3 is tolerant to *Powdery Mildew.*

Powdery Mildew Isolate KP4 UGM Characteristics

Infection spots under microscope (10 μm and 40 μm) (Figure 2a-b) showed that *Powdery Mildew* isolate of KP4 UGM is classified to the genus Podosphaera and characterized by the presence of true chains, combination of conidium. The first appearance of *Powdery Mildew* infection isolate KP4 can be found on the oldest leaves. In addition, the pathogen infection rate were worse in older leaves. On young melon leaves, ≥2 weeks old, smaller numbers of conidia were found germinated. Furthermore, the symptom on the leaves showed discoloration on the infected leaves from bright green to yellowish (chlorosis) and followed by death of leaves.

The Appearance and Phenotypic Characteristics of Melons Infected with *Powdery Mildew*

Phenotypic characteristics of melon fruit infected with *Powdery Mildew* (Fig. 3 and Table 2) indicate that pathogen infections have severe influence on the shape of fruit, net density, and the level of sensitivity on the fruit including the primary color of mesocarp, texture, flavor, and aroma. The results of the study revealed that melon cultivars MG3 and Glamour had superior phenotypic characteristics compared to Action 434, MAI, and Ladika cultivars.

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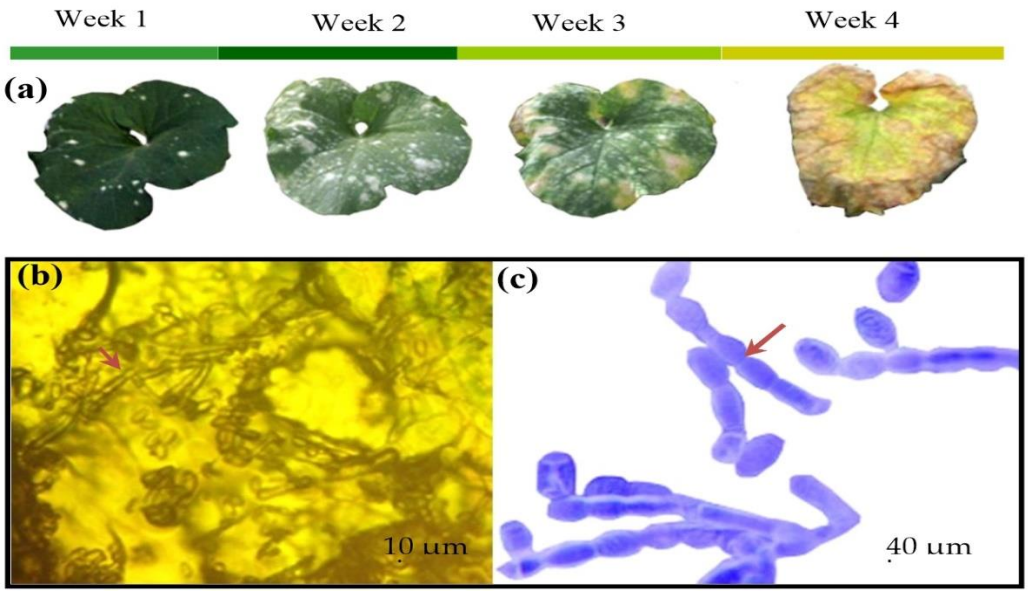

Figure 2. Morphological appearance of *Powdery Mildew*-infected MAI melon leaves (a) at weeks 1, 3, 6, and 8. (b) Microscopic observation of *Powdery Mildew* at 10 µm magnification; (c) and 40 µm (f). Arrows indicate true chain conidia (a feature of the genus Podosphaera).

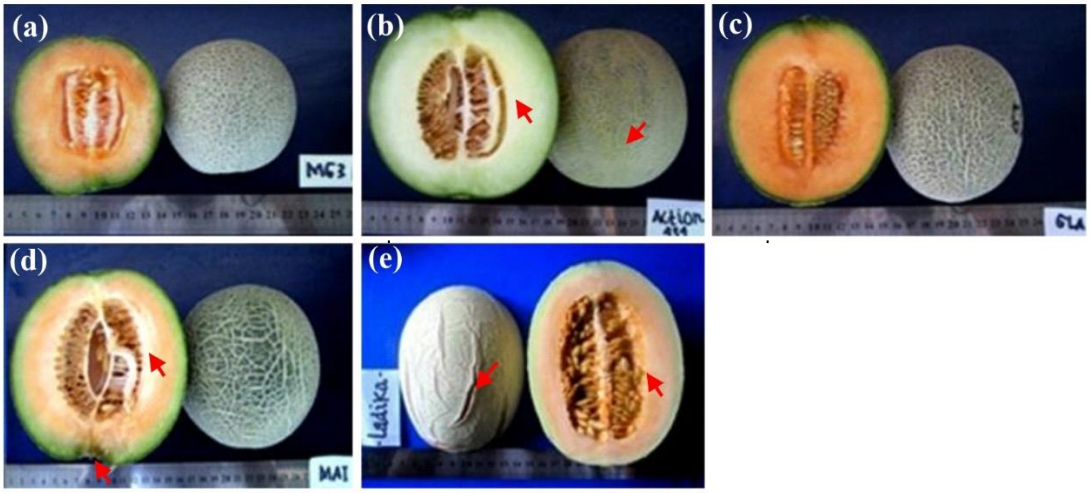

Figure 3. Morphological appearance of melon fruit cultivar (a) MG3, (b) Action 434, (c) Glamour, (d) MAI and (e) Ladika is infected with *Powdery Mildew* . The arrow sign indicates uneven meat (b, d, e), uneven skin colour and broken net (b and e), rottenness on the base (d).

The fruit characteristics of the cultivar MG3 and Glamour were perfect round and oval shape, uniform orange color of the mesocarp, adequate net density (9), sweet flavor (4), and fragrant (4). Furthermore, cultivar Action 434 showed round shape of the fruit with yellow-green color on the skin of the fruit, sub-par net density(5), predominant color

of whitish green mesocarp which indicates that the fruit was not evenly ripe and bitter-sweet taste (3) with weak aroma. (2). Meanwhile, cultivar MAI were as follows: round shape, yellowish green skin color, sub-par net density (7), yellow mesocarp with a white center that indicates uneven ripeness with firm mesocarp gradually softened to the center of the fruit and (1) unpleasant aroma and flavor (1). Lastly, Ladika were: elliptical with yellow skin, sub-par net density (1), yellow mesocarp with firm mesocarp gradually softened to the center of the fruit (rotten), the taste is not sweet (1) and unpleasant aroma and flavor (1).

Table 2. Phenotypic characters of melon fruit cultivars MG3, Action, Glamour, MAI and Ladika infected with *Powdery Mildew*

Types of melons	Phenotypic characters				
	Net density (1-10)	The color of meat	Fruit ripening	Flavours (1-5)	Smell (1-5)
MG3	9	Orange	ripe	4	4
Action 434	5	Whitish green	Less ripe	3	2
Glamour	9	Orange	ripe	4	4
MAI	7	Yellowish-green	Not ripe	1	1
Ladika	2	Light yellow	Not ripe	1	1

Discussion

The response of each cultivar on *Powdery Mildew* was different. In the first week, the leaves of MG3 infected with the isolate of KP4 showed no symptoms. However, in the second week, slight symptoms appeared, but the growth and development of the pathogen was quite slow. In cultivar Action 434 and Ladika, the response to the pathogen infection was similar, the infection spread rapidly across the leaf surface and then infected other leaves. After spreading, the leaf's color began to change from a vibrant green to a yellowish hue (chlorosis), and finally died. There were similarities in the Glamour and MAI in response to the pathogen infection (Fig. 2). The symptoms did not evenly spread on the leaf surface, but the growing colony spread across the surface. Leaves that have already suffered from chlorosis or cell death in the portion infected by the fungus were characterized by the presence of yellow spots, which later turn brown and die. Once the infected part has turned yellow or brown, the infection starts to spread to the healthy leaves and older leaves perish more easily than the younger ones. Resistance of Glamour against pathogen gradually outperformed MAI.

Powdery Mildew spread optimally on six-week-old leaves (Fig. 2). The growth rate of conidia was also strongly affected by environmental temperature. Conidia will grow and thrive at a maximum temperature of 25^0C and cannot grow and germinate at temperatures below 15^0C or above 30^0C. Temperature measurements at *greenhouse* B KP4 UGM showed that the average temperature in the morning (at 07:00 AM) is 29^0C, during the day (01:00 AM) 36.4^0C, and in the evening (at 3:00 PM) 33.6^0C, thus the growth of pathogen was most potential at night and the least in the morning, There was no growth during the day and afternoon in the *greenhouse* B KP4 UGM.

Based on the phenotypic characteristics of the fruit (Fig. 3 and Table 2), each variety of melon responded differently to *Powdery Mildew* infections. Melon cultivar MG3 was tolerant to pathogen infection, and the fruit had good phenotypic characteristics, while the Glamour rate of fungal infection was not excessively high so that the fruit also had good phenotypic character. Melon cultivar Action 434 was highly susceptible to *Powdery Mildew* infection and the fruit was also high in quality based on its net density, flavor, texture, and lack of fruit odor. On the other hand, MAI and Ladika's reaction to *Powdery Mildew* infection on their leaves differed on each cultivar but both were vulnerable to the *Powdery Mildew* and the fruits were subpar with low net density, uneven coloration on the meat, and firm mesocarp texture with a putrid interior, and strong odor.

CONCLUSION

Melons cultivar MG3 was tolerant to *Powdery Mildew* and had a higher percentage of resistance than the remaining four commercial melons, as followed: Action 434, Glamour, MAI, and Ladika. The appearance of infected fruit on MG3 and Glamour were superior compared to cultivar Action 434, MAI, and Ladika. *Powdery Mildew* isolates KP4 UGM belongs to the genus Podosphaera.

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