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Short Communication

First Report and Yield Reduction of Emerging Yellow Spot Disease on Melon (*Cucumis melo*) Caused by Melon Yellow Spot Virus (MYSV) in Indonesia

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ABSTRACT

Typical symptoms of melon yellow spot disease include leaf curling, mosaic, chlorotic spots, fruit discoloration, and cracking, which are constantly found in melon greenhouses in Indonesia. These symptoms lead to considerable losses, reducing fruit weight by up to 66.67% and lowering the Brix score, making the fruit unmarketable. RT-PCR targeting the N-gene of MYSV showed all samples were positively infected. Bioinformatics analysis revealed that Asian isolates of MYSV are highly identical and share a common ancestor, highlighting MYSV as an emerging disease to melon production across Asia.

Keywords: Emerging disease, melon, MYSV, RT-PCR, yield reduction

INTRODUCTION

Melon (*Cucumis melo*) is one of the important cucurbit crops in the world. About 70% of melon productions are in Asia. Indonesia is one of the melon producers in Asia with annual production reaching up to 118,696 tonnes (BPS-Statistics Indonesia, 2022). In the last three years, melon production in Indonesia has decreased by more than 14% due to the high prevalence of plant diseases caused by various pathogens. Viruses account for the most anticipated pathogens for melon due to their rapid transmission and significant losses of up to 100%. There are several virus genera known to be associated with melon diseases in Indonesia, namely *Potyvirus,*

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Tobamovirus, Cucumovirus, Begomovirus, *Comovirus*, and *Orthotospovirus* (Adachi-Fukunaga et al., 2020; McLeish et al., 2022).

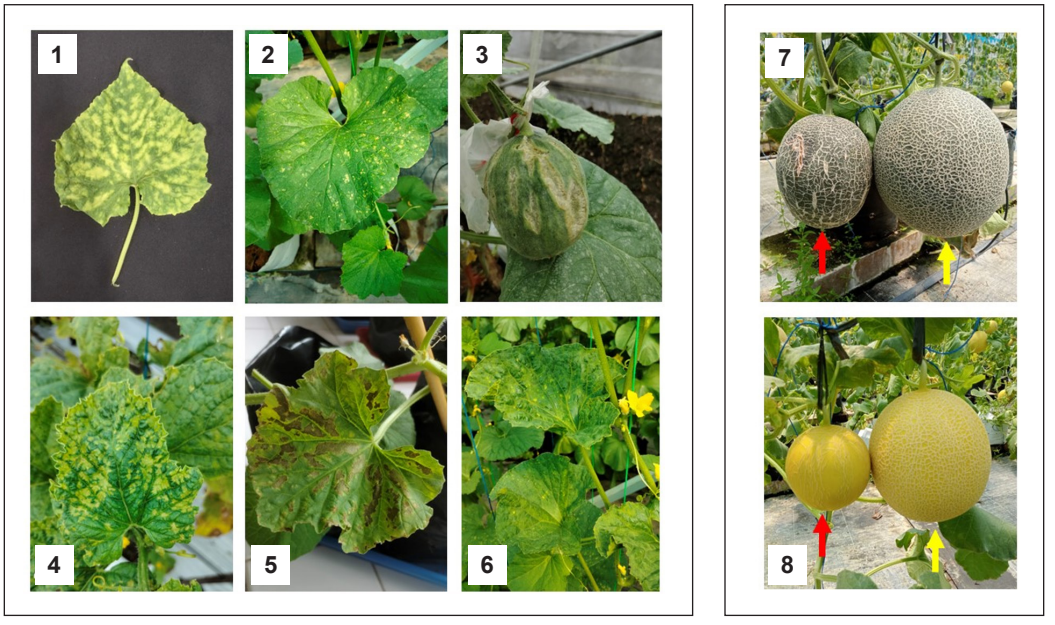
Yellow spot disease is currently emerging in Asia and is becoming a major threat to melon cultivation. It is caused by melon yellow spot virus (MYSV) (Chen et al., 2010). MYSV is a member of the *Orthotospovirus* genus. It was known persistently transmitted by thrips and was first reported in Japan (Adachi-Fukunaga et al., 2020; Chakraborty et al., 2018; Kato et al., 2000). Later, MYSV was found in several countries including China (Gu et al., 2012; Sun et al., 2020), Taiwan (Peng et al., 2011), Thailand (Chiemsombat et al., 2008; Supakitthanakorn et al., 2018), Ecuador (Quito-Avila et al., 2014), and India (Pradeep et al., 2024). The symptoms of MYSV infection are chlorotic spot, mosaic, leaf curl, fruit discoloration, and fruit cracking resulting in production failure and total economic loss due to unmarketable products. Compared to established viral infections like Begomovirus, Orthotospovirus infections have the potential to become epidemics in the future. This is due to the wide host range, high genetic diversity, persistent transmission by thrips, and the lack of resistant sources (Pradeep et al., 2024).

During the 2021-2023 survey in several melon production greenhouses in Indonesia, we constantly found melon plants exhibiting symptoms including leaf curling, leaf mosaic, chlorotic spots, necrotic spots, fruit discoloration, and fruit cracking with incidence reaching up to 90% in melon population (Figure 1). These symptoms appear simultaneously with the high thrips (*Thrips parvispinus*) population, resembling typical orthotospovirus infections. These symptoms followed by a high thrips population have never been found before in Indonesia. Considering the significant importance of the disease for melon cultivation and developing management strategies, this research aimed to determine the causal agent of the disease and evaluate the damage to melon cultivation. We carried out molecular detection targeting several common viruses on melon including *Tobamovirus, Potyvirus, Begomovirus*, and MYSV. However, of all tests carried out, only MYSV detection showed positive results, indicating that the yellow spot disease on melon is caused by MYSV. This finding suggests that yellow spot disease caused by MYSV is a novel pathogen in Indonesia and poses a serious threat to melon cultivation.

MATERIALS AND METHOD

Sample Collection

Melon leaves showing yellow spot symptoms were collected from four greenhouse locations in Central Java: Solo, Bergas, Yogyakarta, and Tegal. Leaves of the diseased plants were collected in purposive sampling. Samples collected from the plant showed leaf mosaic, leaf spot, leaf chlorosis, and leaf necrotic spot in the early generative stage. The early generative stage is the critical time of plants from thrips infestation. Samples were documented, stored dry, and subsequently used for RT-PCR.



(a) (b)

Figure 1. (a) Symptom variations of MYSV in melon plants; and (b) size comparison between fruits. Symptom variations could be a leaf mosaic (1), yellow spot (2), fruit cracking (3), leaf curling and mosaic (4), leaf necrotic (5), or leaf cupping (6). Fruit from diseased plants (7 & 8 red arrows) are smaller, cracked, and might have uneven nets compared to the healthy one (7 & 8 yellow arrow)

Field Observation and Damage Evaluation

Two greenhouses located in Solo and Bergas were utilized to model yield losses in melon due to disease. Each greenhouse housed an estimated 3,000-4,000 plants. Disease impact was assessed by evaluating several parameters: disease incidence, severity, fruit weight loss, and fruit Brix. Disease incidence was determined by calculating the proportion of diseased plants within the total plant population in each greenhouse. Disease severity was assessed by quantifying the number of symptomatic leaves on each plant relative to the total number of leaves. Fruit weight loss was determined by comparing the weight of fruits from diseased plants to those from healthy plants. Finally, fruit Brix was measured using a refractometer.

RNA Extraction and RT-PCR

Samples were subjected to RT-PCR. Total RNA extraction was performed using the Total RNA Mini Kit (Geneaid, Taiwan) according to the manufacturer's instructions. cDNA synthesis was performed using a Revertra-ace cDNA synthesis kit (Toyobo, Japan) with an n-hexamer primer. cDNA was subsequently used as a PCR template. PCR was performed in a total 50 µL reaction consisting of 25 µL MyTaq HS Red Mix 2X (Meridian, USA),

2 µL each forward and reverse primers (10 pmol/µL), 19 µL nuclease-free water, and 2 µL cDNA (10 ng/µL) as template. The primers used to target the nucleocapsid (N) gene of MYSV (Charlermroj et al., 2017). The PCR conditions were pre-denaturation at 95°C for 3 minutes followed by 35 cycles of denaturation at 95°C for 1 minute, annealing at 55°C for 30 seconds, extension at 72°C for 1 minute, and final extension at 72°C for 10 minutes. PCR products were visualized by 1% agarose electrophoresis and subjected to bidirectional Sanger sequencing.

Bioinformatics Analysis

Nucleotide sequence data was analysed using BLAST (https://blast.ncbi.nlm.nih.goc/Blast.cgi/) and subsequently deposited in Genbank (Accession No. OR405986-0R405989). Nucleotide alignment was performed using ClustalW. The phylogenetic tree was constructed using MEGA 7 with Neighbor-Joining Method and 1000 bootstraps replication (Kumar et al., 2016).

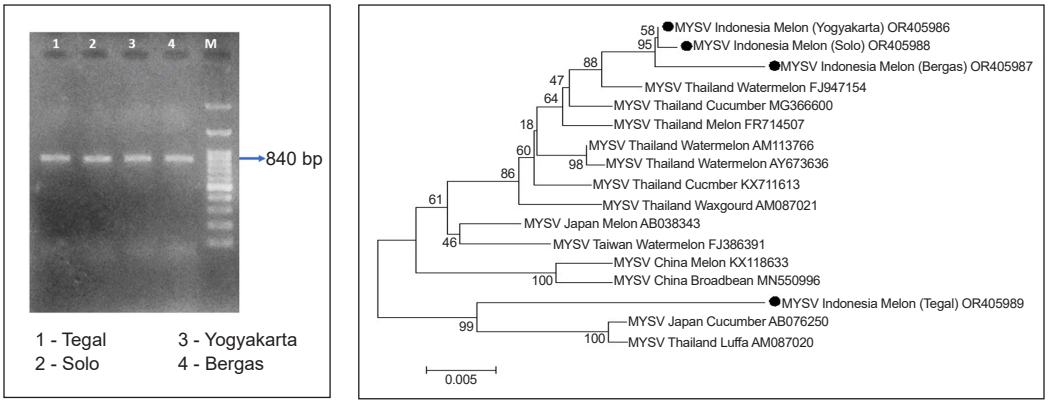
RESULTS

Field observations revealed characteristic MYSV symptoms including leaf cupping, dark green spots on leaves, and vein yellowing. These symptoms specifically appeared during the early generative phase, from the onset of flowering until fruit set (Figure 1). The early generative phase, particularly during flower formation, is a highly preferred phase for thrips (Ren et al., 2020).

The data showed disease incidence can reach up to 85%, indicating that MYSV is prevalent in these locations. Comparison between healthy plants vs diseased plants showed that the disease caused significant weight losses up to 66,67% and significantly lower average Brix score (9 vs 13 on healthy plants) (Table 1). The lower weight and Brix score on diseased plants consequently caused the fruit not to meet the market standard. Moreover, it resulted in total economic losses.

Table 1
Field observation data on two greenhouses

Greenhouses	Population (plants)	Status	Severity (%)	Incidence (%)	Fruit Weight (gr)	Brix
Solo	4000	Healthy	0	0	1500	13
		Diseased	50	85	500	9
Total loss percentage			0	0	66,67%	30,71%
Bergas	3500	Healthy	0	0	1350	13
		Diseased	35	70	550	9
Total loss percentage			0	0	59,26%	30,71%



(a) (b)

Figure 2. (a) PCR Visualization; and (b) phylogenetic diagram of samples obtained from Tegal (1), Solo (2), Yogyakarta (3), and Bergas (4). The phylogenetic tree of partial N-gene of MYSV using the Neighbor-Joining method with 1000 bootstraps replication showed MYSV isolates in Asia are closely related to each other

All samples tested for MYSV resulted in expected bands of approximately 840 bp (Figure 2). BLASTn search results of nucleotide sequences showed 96-99% identity with MYSV from Japan and Thailand from various host sources. A phylogenetic tree constructed from partial N-gene of MYSV showed Asian isolates are clustered in a single branch indicating these isolates share a common ancestor (Figure 2). These results indicate that the MYSV isolates spread in Asia are closely related. To our knowledge, this is the first report of MYSV in Indonesia.

DISCUSSION

The existence of MYSV in Indonesia poses a serious threat not only to melon cultivation but also to other cucurbitaceae cultivation in general. MYSV was also known to infect several crops including cucumber, watermelon, pumpkin, balsam pear, and chili pepper (Sunpapao, 2012; Supakitthanakorn et al., 2018; Takeuchi et al., 2009). This shows that the potential for disease caused by MYSV in horticultural crops is quite large in the future.

Our study indicates that the MYSV detected in Indonesian melons likely shares a common ancestor with MYSV isolates found in other Asian hosts, suggesting potential cross-transmission among various plant species, including weeds (Yamasaki et al., 2012). Further distribution of MYSV might exacerbated by common agricultural practices in Indonesia, particularly open-field intercropping. Open-field systems allow the movement of insect vectors between primary crops and weeds, which can serve as alternative virus hosts. While MYSV is primarily transmitted by *Thrips palmi* (melon thrips), it is hypothesized that MYSV might also be transmitted by other thrips species, such as *T. javanicus, T. tabaci,* or *T. parvispinus*. *Thrips parvispinus* has become a dominant species in Indonesia, replacing *T. palmi* (Murai et al., 2010; Sartiami & Mound, 2013).

The MYSV transmission is exclusively facilitated by thrips in a persistent manner. Thrips is also known as cosmopolitan insects with a broad host range. The widespread presence of thrips and their ability to transmit MYSV across different plant species underscore the need for comprehensive management strategies to control the viral spread. (Chakraborty et al., 2018; Peng et al., 2011).

The existence of MYSV as a novel pathogen in Indonesia adds to the long list of diseases in Cucurbitaceae. Thrips as the exclusive vector of MYSV have a short life cycle, reproduce by parthenogenesis, and are quickly resistant to insecticides (Wakil et al., 2023). Up to now, thrips are known resistant to classes of insecticides including organochlorines, organophosphates, carbamates, pyrethroids, and spinosyn (Negash et al., 2020). Until now, there are no known effective chemical pesticides to control thrips (Gao et al., 2012). Meanwhile, control measures using traps, net houses, and biological agents are so far not known to have a significant impact. Therefore, thrips control must be carried out with combined measures in an IPM framework.

To effectively control the spread of MYSV, a comprehensive approach is essential, including the eradication of diseased plants, vector management, and greenhouse sanitation. Thrips, as the primary vector of MYSV, play a crucial role in the virus transmission, thus, the control of thrips is the key to disease management. Simultaneously, the eradication of infected plants minimizes the inoculum source, preventing further viral propagation. Implementing strict greenhouse sanitation practices, such as regular cleaning and disinfection, reduces the risk of MYSV transmission by eliminating potential breeding grounds for thrips and other pests.

MYSV is expected to emerge as a significant pathogen of cucurbits shortly due to its persistent transmission by thrips and its ability to infect a broad range of host plants. The absence of commercially available melon cultivars with resistance to tospoviruses further underscores the urgent need to develop resistant varieties. This challenge is particularly critical for horticultural breeding programs focused on cucurbits. The insights gained from this research are expected to play a key role in shaping effective MYSV control strategies and guiding the development of tospovirus-resistant melon cultivars, thereby safeguarding future melon production. Further research should focus on the transmission mode and ecological aspect of virus-vector relationships.

CONCLUSION

The occurrence of yellow spot disease in melon caused by MYSV poses a serious threat to melon cultivation in Indonesia. The disease caused significant losses in fruit weight and decreased fruit quality, resulting in unmarketable fruits and total economic loss. To the best of our knowledge, this is the first report of MYSV and its losses in Indonesia.

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