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Virus Identification in Yard Long Bean Plants with Yellow Mosaic Symptoms

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ABSTRACT: Begomovirus infection, which causes yellow leaf curl disease, is commonly found in the Bengkulu province, affecting various plants, including chilli, melon, cucumber, papaya, and weeds. The viruses reported to date in long bean plants include *Mungbean yellow mosaic India virus* (MYMIV) and *Bean common mosaic virus* (BCMV). This study aimed to detect viruses in long bean samples collected from Musi Rawas (South Sumatra) and North Bengkulu (Bengkulu), areas where yellow mosaic symptoms resembling those caused by Begomovirus infection were observed. Virus detection was performed using PCR-based DNA analysis with specific MYMIV (MY1/MY2) primers, targeting a DNA fragment of approximately 238 bp. The results revealed that yard-long bean plants from Musi Rawas were positively infected with MYMIV, while those from North Bengkulu tested negative for MYMIV. Begomovirus or other viral infections may cause the yellow mosaic symptoms observed in North Bengkulu yard long beans. Therefore, further investigations using additional primers are needed to identify the specific virus responsible for the symptoms in this region.

Keywords: mungbean yellow mosaic India virus; polymerase chain reaction, symptom

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INTRODUCTION

Yard-long bean plants are known to be susceptible to infections by various viruses. In Java, yellow mosaic disease in long beans has been identified as being caused by *Bean common mosaic virus* (BCMV) (Damayanti et al., 2009) and *Mungbean yellow mosaic India virus* (MYMIV) (Nurulita et al., 2015). Yellow leaf curl disease, caused by viral infections, is commonly found in cultivated crops and weeds. The characteristic yellow mosaic and leaf curl symptoms on plants are typical of *Geminivirus* infections.

Geminivirus has a single-stranded circular DNA genome, with fragment lengths ranging from 2,500 to 5,200 base pairs. The Geminivirus family consists of several genera, including Begomovirus, which is transmitted by whiteflies; Curtovirus, Grablovirus, Mastrevirus, and Turncurtovirus, which are transmitted by planthoppers; and Capulavirus, transmitted by aphids (King et al., 2012). Among these genera, *Begomovirus* is the most species-rich, with over 320 species that infect a wide range of hosts (Zerbini et al., 2017). Notable *Begomovirus* species include *Mungbean yellow mosaic India virus* (MYMIV), *Pepper yellow leaf curl virus* (PYLCV), *Bean golden mosaic virus* (BGMV), *Tobacco leaf curl virus* (TLCV), and *Tomato yellow leaf curl virus* (TYLCV) (King et al., 2012).

In Bengkulu, several *Begomovirus* species have been reported infecting crops such as papaya (Sutrawati et al., 2021) and chilli (Aulia et al., 2022; Sipriyadi et al., 2022; Sutrawati et al., 2022). The first report of *Begomovirus* infection in long beans came from West Java, Central Java, and Yogyakarta in 2015, with the viruses identified as MYMIV and BCMV (Nurulita et al., 2015; Melinda et al., 2015). MYMIV was also detected in soybean plants in Cirebon, Bantul, and Musi Banyuasin (Sutrawati et al., 2020), as well as in long beans in Sleman, Yogyakarta,

where it caused yellow mosaic symptoms on the leaf lamina (Pertiwi et al., 2021). A mixed infection of multiple *Begomovirus* species in long beans was first reported as an outbreak in Bogor in 2009, with an incidence rate of 100%, leading to a significant decline in harvest quality and even plant death (Damayanti et al., 2009).

Yellowing and curling of the leaves in long bean plants have also been observed in Bengkulu and South Sumatra. Damage and potential yield loss in yard-long beans caused by Begomovirus infection should be anticipated by establishing detection methods and disease control strategies. Basic information regarding the molecular and biological characteristics of the virus is required. Therefore, it is essential to conduct research to investigate the cause of yellow mosaic disease in long beans in this region. Virus detection will be carried out using molecular techniques based on nucleic acids. This method offers high sensitivity and greater accuracy compared to traditional serological virus detection methods (Anggraini & Hidayat, 2014).

MATERIALS AND METHODS

This study was conducted from July to October 2023. Sampling was conducted in the Awidodo, Musi Rawas Regency, South Sumatra, and Sumber Agung, North Bengkulu Regency, Bengkulu. The samples were subsequently analyzed at the Molecular Biology Laboratory for Plant Protection, University of Bengkulu.

Detection of Virus using Specifics Primer for MYMIV.

Total DNA Extraction

Total DNA extraction was performed using the CTAB (Cetyl Trimethyl Ammonium Bromide) method, based on the protocol outlined by Doyle & Doyle (1987). Young leaves from long bean plants were weighed (0.1 grams) and ground into a fine powder using a mortar and pestle in liquid nitrogen. The powdered leaf tissue was then transferred to a 2 mL Eppendorf tube and 500 µl of CTAB buffer was added. The mixture was vortexed to ensure homogenization, followed by incubation at 65°C for 60 minutes.

After incubation, the Eppendorf tube was allowed to cool at room temperature for about 2 minutes, and 500 µl of chloroform: isoamyl alcohol (24:1 v/v) was added. The solution was mixed gently and centrifuged at 12,000 rpm for 15 minutes. The supernatant (300 µl) was transferred into a new 2 mL Eppendorf tube. Sodium acetate (CH3COONa) was added at 1/10 of the supernatant volume, and isopropanol was added at 2/3 of the supernatant volume. The mixture was incubated overnight at -30°C.

The next day, the sample was centrifuged at 12,000 rpm for 10 minutes. The supernatant was discarded, and the DNA pellet adhered to the wall of the tube. The DNA pellet was washed with 500 µl of 70% ethanol and centrifuged at 8,000 rpm for 5 minutes. After discarding the ethanol, the pellet was air-dried. Finally, 50 µl of nuclease-free water was added to dissolve the DNA. The total DNA was stored at -20°C for future use.

DNA Amplification

DNA amplification was performed using specific primers for MYMIV: MY1 (5'-TTACATGGTCCCTCGCAACC-3') and MY2 (5'-ACAGCCTTCTCTACCCCGAT-3'), targeting a fragment of approximately 238 bp. The PCR reaction mixture included 1 µl of DNA template, 0.5 µl of each primer (MY1 and MY2), 4.75 µl of nuclease-free water, and 6.25 µl of Green Taq DNA Polymerase.

The PCR program consisted of the following steps: Pre-denaturation: 94°C for 5 minutes, denaturation: 94°C for 1 minute; annealing: 61°C for 1 minute; Extension: 72°C for 1 minute; final extension: 72°C for 10 minutes. The amplification was repeated for 35 cycles (Nurulita et al., 2023).

DNA Visualization

The amplified DNA was visualized using 1% agarose gel electrophoresis in a Tris-Acetate EDTA (TAE) buffer (0.5x). The gel was then visualized using a gel documentation system (Axygen).

RESULTS AND DISCUSSION

Symptoms of Virus Infection

Incidence of yellow mosaic disease was high i.e. 30-50% in yard long bean growing areas. Infected plants were recognized in the field based on visual symptoms. Two main symptoms of yellow mosaic disease were observed, i.e. mosaic vein banding (figure 1A) and yellowing with

green spots (figure 1B). Yellowing with a green spot was thought of as an early symptom before it developed into yellowing (Nurulita et al., 2015). The mosaic-like pattern of bright and dark green patches on the leaves results from virus multiplication in the plant, destroying the already present chlorophyll. Because of the active replication process, viral infections also cause an increase in plant respiration and decrease the amount of carbohydrates in plant tissues (Agrios, 2005). Long bean plants in Jawa, West Jawa, and Central Jawa have shown similar signs of mosaic and yellowing (Nurulita et al., 2015). MYMIV-infected long beans in Sleman Regency exhibit signs such as leaf twisting, mosaic patterns, and yellowing (Pertiwi et al., 2021). In Central Java, several virus species have been found in long bean plants, including *MYMIV*, *Bean common mosaic potyvirus* (BCMV), and *Cucumber mosaic virus* (CMV). Mixed infections in long beans result in yellow mosaic symptoms on the leaf lamina (Supyani et al., 2020). Purwaningsih et al. (2016) reported that a single infection of *MYMIV* causes yellowing symptoms, while a single infection of *BCMV* results in mosaic symptoms on long bean plants.

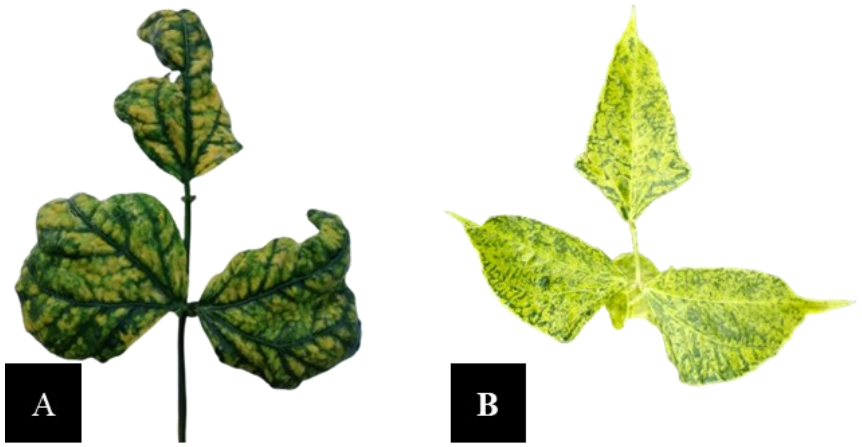

Figure 1. Symptoms on long bean plants; Vein banding (A) and Yellowing (B)

Detection of Viruses Using Specific Primer for MYMIV

According to PCR analysis, yard-long bean leaf samples from Musi Rawas (South Sumatra) contained a DNA band of around 238 bp, which corresponded to the target of primers MY1/MY2. However, North Bengkulu (Bengkulu) samples did not show any MYMIV DNA band (Figure 2). These findings demonstrate that, whereas the samples from North Bengkulu tested negative for MYMIV, the long bean leaf samples from Musi Rawas are positively infected with the virus.

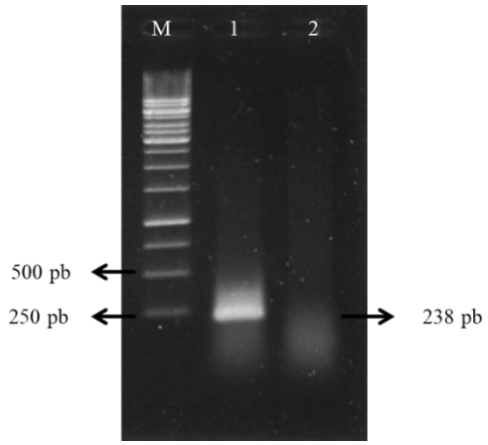

Figure 2. DNA Visualization Results Using MYMIV-Specific Primers Note: M: 1 kb Marker; 1. South Sumatra; 2. Bengkulu sample.

Although the long bean samples from Bengkulu exhibited yellowing symptoms similar to those typically associated with *MYMIV* infection, the PCR results were negative. This suggests that the long bean plants from Bengkulu may be infected with a different virus. This could be due to the fact that different viruses can cause similar symptoms (Agrios, 2005). In long bean plants, yellow mosaic disease can also be caused by *BCMV* and *CMV* infections (Damayanti et al., 2009).

MYMIV belongs to the *Begomovirus* group, which is transmitted by the whitefly vector (*Bemisia tabaci*) in a persistent manner. The whitefly only needs one acquisition period to be able to transmit the virus for the rest of its life (King et al., 2012). In addition to vector transmission, *MYMIV* can also be seed-borne, with the virus transmitted through seeds from infected plants. Seed transmission of *MYMIV* can be detected in seed parts such as the seed coat, cotyledons, and young leaves (Mulyadi et al., 2021).

The host range of *MYMIV* is quite broad. It can infect a variety of crops, including long beans, mung beans, cucumbers, peppers, eggplants, and tomatoes, with characteristic yellow mosaic symptoms (Mulyadi et al., 2021). In long beans, *MYMIV* infection significantly affects crop yields, resulting in empty pods and fewer pods produced (Purwaningsih et al., 2016).

MYMIV infection has been reported to cause significant crop losses in several countries. For instance, *MYMIV* caused a 70–100% disease incidence in *Phaseolus vulgaris* in Pakistan (Naimuddin et al., 2011) and 70–80% in *Vigna mungo* in Nepal (Shahid et al., 2012). *MYMIV* has also been reported to infect *Phaseolus vulgaris* in Oman (Shahid et al., 2016). In Indonesia, *MYMIV* was previously reported to cause damage to long beans in various regions of Java (Nurulita, 2015). Additionally, *MYMIV* has been reported in soybeans in Brebes (Tsai et al., 2013) and other regions, including Bantul, Cirebon, and Musi Banyuasin (Sutrawati et al., 2020). These findings indicate that *MYMIV* is widespread across Java and beyond, with a broad host range, and poses a potential risk to crop yields, making it important to monitor and manage the virus to prevent further losses.

Several strategies can be employed to control virus diseases, such as controlling insect vectors, managing weeds that may serve as alternative virus hosts, using virus-resistant varieties, and ensuring the use of virus-free seeds. Another potential approach is the induction of plant resistance to virus infections. Plant extracts containing ribosome-inactivating proteins (RIPs) can inhibit viral replication (Verma et al., 1998). These plant extracts also serve as agents to enhance systemic acquired resistance in plants (Deepthi et al., 2007).

CONCLUSION

Molecular detection using a specific primer for MYMIV confirmed the association of MYMIV in yellow mosaic disease on yard-long beans. Further study on disease spread, host range, and insect transmission is very important to understanding disease development and control.

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