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Molecular Identification of *Mungbean yellow mosaic virus* Infecting Yard Long Beans and *Aclypha indica* in Kediri, East Java

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ABSTRACT

Mungbean yellow mosaic virus (MYMV) is a Begomovirus species that causes yellow disease in legumes, particularly yard-long beans. *Acalypha indica* (Euphorbiaceae) is a weed that thrives in yard-long beans fields. Information on these weed species that are naturally infected is needed so that they can serve as MYMV reservoirs. This study aims to determine the MYMV's symptoms, molecular evidence, and diversity. Purposive sampling was used to obtain leaf samples, followed by molecular detection using specific primers, phylogenetic analysis with BioEdit v.7.0.5, and MEGA X version 10.2.6 based on the Neighbor-Joining (NJ) approach. In Kediri, the incidence of yellow mosaic diseases on yard-long beans is nearly 100%, with systemic symptoms such as yellowing, yellow mosaic, mosaic with a yellow-green pattern, leaf malformation, and stunting. Mosaic symptoms are present in several *A. indica*. The CTAB method was used to isolate total DNA, yielding a concentration of ±100 ng with λ DNA as a comparison. Specific primers (AC2-F & AC2-R) have successfully amplified MYMV in yard-long beans and *A. indica*. Phylogenetic analysis revealed that the MYMV from Kencong was most closely related to the *Mungbean yellow mosaic India virus* (MYMIV) from soybean-Brebes and cowpea-Purwakarta. The phylogenetic tree shows bootstrap value in 94 of 1000 iterations between isolates and in a single cluster.

Keywords: Begomovirus, euphorbiaceae, leguminosae, MYMV, PCR, specific primer, weed-associated

Introduction

Vegetable commodities have a great potential to meet people's nutritional needs. Yard long beans, *Vigna unguiculata* subsp. *sesquipedalis*, are widely cultivated legumes that are commonly consumed as green vegetables (Damayanti et al., 2009). As a result, efforts are made each year to increase planted area and productivity. Although the harvested area of long beans in East Java has increased year after year, production has decreased by 6.1%, from 45,015 tons/ha in 2016 to 39,878 tons/ha in 2021 (BPS, 2022). Infection with pathogens is one of the constraints limiting plant production. *Mungbean yellow mosaic virus* (MYMV) is a Begomovirus virus that causes yellow diseases in yard-long beans (Ilyas et al., 2010). The whitefly *Bemisia tabaci* (Gennadius) transmits it in a circularly persistent manner (Hemiptera: Aleyrodidae) (Hunter et al., 1998; Ghanim & Czosnek, 2000; Su et al., 2014).

Infection with Begomovirus species in Leguminosae plants is no longer a new disease. The yellow disease was previously reported to be widespread in several areas of West Java between

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2008 and 2009 (Damayanti et al., 2009); yard-long beans infections in Bogor, Purwakarta, and Brebes were reported in 2009 (Tsai et al., 2011). Yellowing, vein banding (thickening of the leaf bones), mosaic with green spots (Nurulita et al., 2015; Mulyadi et al., 2021), and mosaic symptoms on the pods (Mulyadi et al., 2015) are all symptoms of infection in infected yard long beans.

MYMV, which infects yard-long beans, is a viral species with a diverse range of hosts, including legumes and weeds. The role of sources of inoculum around the plantation, both cultivated plants and weeds, is one of the triggers for the high distribution of infection, according to Aidawati et al. (2005) weeds and other cultivated plants around the plantation can potentially be a source of inoculum. Previous research has only focused on plant cultivation and has not investigated the relationship between local weed infections. It has previously been reported that in India, an earring weed (*Acalypha indica*) was found to be infected with yellow diseases (Rani et al., 1996; Mall et al., 2014; Mishra et al., 2010). Weeds were found to grow throughout the growing season in yard-long beans, according to survey results. Given the scarcity of information on the presence of MYMV, which infects yard-long beans and earring weeds, particularly in Kediri, molecular testing using PCR (*Polymerase Chain Reaction*) is required. As a result, the goal of this study was to identify MYMV symptoms, molecular evidence, and diversity.

Material and Methods

Fieldwork and sample collection

The study was carried out in stages, including field surveys, sample collection, and molecular detection of MYMV. Field surveys and sample collection of yard-long beans and *A*. *indica* weeds were conducted in Kencong, East Java, while MYMV detection was performed at the Plant Protection and Molecular Breeding laboratory of PT. BISI International Tbk. in Pare, Kediri Regency, East Java.

Purposive sampling is used for leaf sample collection, and the sample unit collected is representative of subjects who meet predetermined criteria (Morissa, 2012). The criteria are leaf malformation, mosaic symptoms, yellowing, and stunting. Leaf samples were sealed in airtight plastic and kept in a freezer at -80ºC.

Molecular detection of Mungbean yellow mosaic virus

Total DNA is extracted from plant samples using the CTAB method (Doyle & Doyle, 1990) with a slightly modified, the pellet is dried in an oven at 60ºC. Electrophoresis of DNA extracted from the genome was used to determine the concentration of the DNA template. The step is 10 µl of DNA (New England Biolabs) was inserted into the first well (functioning as a control). After that, 9 µl of 0.6X loading dye and 1 µl of DNA template were inserted into the next agaros gel well. For 30 minutes, the electrophoresis device was set to 120 volts. The template concentration was determined by comparing the electrophoresis visualization results of the template genome and DNA (10 µl =100 ng).

Total plant DNA extraction was amplified by PCR using MYMV-specific primer pairs, AC2-F (5'-AGCTAATGACCCCTAAATTAT-3') dan AC2-R (5'-GAGTACTTGGA TGAAGAGAAC-3') with a target DNA fragment of ~504 bp (Mishra et al., 2010). Master mix PCR (New England Biolabs) with a composition of 14.5 µl ddH2O, 2 µl 1X PCR buffer + MgCl2 1,5 Mm, 0.3 µl MgCl2, 0.3 µl dNTP mix, @ 0.8 µl Primer F/R, 0.2 µl Taq polymerase, and 1 µ 10-15 ng template. The PCR profile was preceded by an initial denaturation of 94ºC for 4 minutes, followed by 30 cycles of denaturation at 94ºC for 30 seconds, 55ºC annealing for 1 minute, and 72ºC extension or 1 minute, then 72ºC final extension for 1 minute.

PCR products were visualized using a 1.5% agarose gel (7.5 g Agarose and 500 ml TBE 1X), Ethidium Bromide (EtBr) was added in the amount of 5 µl. Then, 1 µl of loading dye 6X (New England Biolabs) and 10 µl of mixed DNA template was added to the agarose gel well, and 5 µl of 1 kb DNA marker (New England Biolabs) were added to the first well. The electrophoresis device was turned on for 60 minutes at a voltage of 150 volts. By looking at the DNA fragments/bands,

DNA can be visualized, and the results are immediately documented in the Gel Documentation (Kodak Molecular Imaging, UK).

Phylogenic analysis of mungbean yellow mosaic virus

Product PCR amplification was done by DNA sequencing at PT. BISI International Tbk using MYMV-specific primers. Homology level analysis among MYMV that found in GenBank using Blast (Basic Local Alignment Search Tool) (www.ncbi.nlm.nih.gov). The phylogenetic tree was constructed using BioEdit v.7.0.5 and MEGA X version 10.2.6 based on the Neighbor-Joining (NJ) approach.

Results and Discussion

Several research findings indicate that the prevalence of disease caused by Begomovirus species ranges from 0-100% in several areas of East Java (Malang and Blitar) (Santoso, 2014). The condition of yard-long beans plantations in Kencong reveals the presence of yellow diseases with a nearly 100% incidence. Yellow diseases caused by MYMV in yard-long beans have a wide range of symptoms, from the common yellow mosaic and leaf malformations to yellowing with green spots and curling (Figure 1). Symptoms of leaf malformation include leaf thickening, reduced leaf size, and curling upwards, which is most common in young leaves. If the symptoms are severe, the curled young leaves will shrink and crackle like blisters, with discoloration to mosaic and dark green or mosaic with a yellow-green pattern, and then stunting. According to Nurulita et al. (2015), MYMV-infected yard-long beans in Tegal, Sleman, Klaten, and Magelang exhibit yellowing and yellow mosaic symptoms.

Figure 1. Symptoms of MYMV associated with yellow diseases on yard-long beans (a) and *Acalypha indica* (b) in Kencong

Several earring weeds (*A. indica*) have been discovered around the yard-long beans, which have been identified as virally infected. Not all the weeds displayed symptoms, with only a few *A. indica* displaying mosaic symptoms (Figure 1). The determination of which weed samples to test becomes more difficult because the symptoms that appear are nearly identical to symptoms of other diseases. This factor may contribute to a scarcity of information about MYMV infection in earring weeds, particularly in East Java. In reality, these weeds have the potential to be a source of inoculum as well as alternative hosts for yellow diseases. The presence of weeds, according to Mishra et al. (2010), facilitates the spread of disease at the start of the growing season. Many reports have shown that weeds serve as alternative hosts for the survival and spread of begomovirus species (Rani et al., 1996; Mall et al., 2014; Mishra et al., 2010; Barreto et al., 2013).

Yellow and green mosaic spots on the leaves are symptoms of *A. indica* weeds infected with MYMV (Mishra et al., 2010).

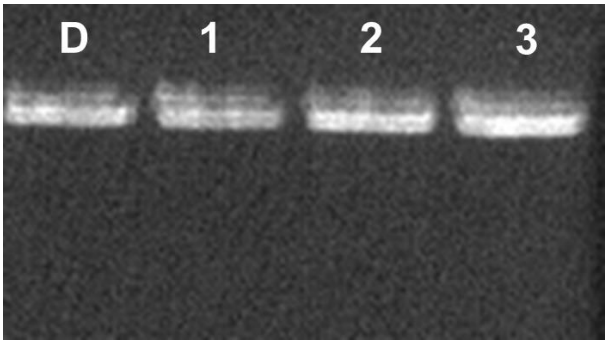

Figure 2. The concentration of Genome DNA through agarose gel electrophoresis. (D) λ DNA (100 ng); (1-3) yard long beans DNA genome

The concentration of template (genome DNA) was determined by comparing the results of electrophoresis visualization of the template genome and DNA (10 µl =100 ng). DNA template to be used for PCR must have a concentration of 10-15 ng, if the electrophoresis results of the genome template have a concentration then diluted with TE buffer pH 8 1X. The concentration of genomic DNA from yard-long beans in Kencong was 100 ng as determined by electrophoresis (Figure 2). The total volume of the diluent made as much as 100 µl, with the addition of 10 µl of DNA template in a 1.5 ml tube containing 90 µl of TE buffer pH 8 1X. Finally, a concentration of 10 ng of template DNA is present in the tub.

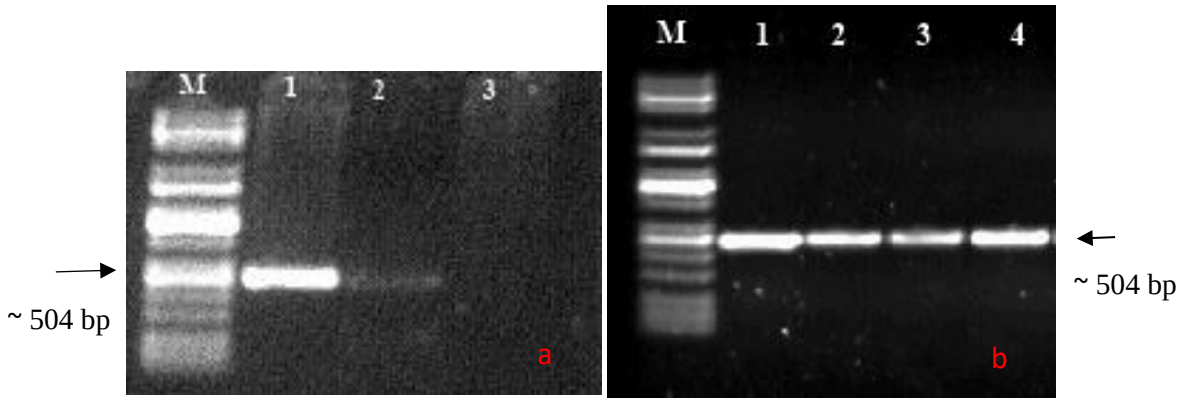

Figure 3. Agarose gel electropherogram of DNA amplified using specific primers (AC2-F & AC2-R); (a) M, 1 kb ladder; 1, yard long beans; 2, *A. indica* with symptoms; 3, *A. indica* with no symptoms appeared (b) M, 1 kb ladder; 1, positive control; 2-4, yard long beans

The presence of a viral infection that causes yellow diseases is detected molecularly using specific primer pairs that will later indicate the type of Begomovirus species that infects. Because the desired target is very species-specific, specific primers are preferred over universal primers for use. The use of specific primers can improve the detection method's efficiency and effectiveness, allowing the species involved to be identified (Ye et al., 2012). In yard-long beans, the primer pair AC2-F & AC2-R successfully amplified the MYMV DNA fragment measuring 504 base pairs (bp) (Figure 3). The results of detection using specific primers will provide molecular evidence of the association of Begomovirus (MYMV) species with yellow diseases in plant samples. The MYMV species is a begomovirus with the main host plant being planted in the Leguminosae family, including yard-long beans.

Figure 3 shows that *A*. *indica* weeds with mosaic symptoms were successfully amplified to a target DNA fragment size of 504 bp, whereas asymptomatic weed isolates were not amplified. The thickness of the target DNA fragments varied (Figure 3a), with the yard-long beans' DNA fragments being thicker than the weed isolates. This can be attributed to the more severe symptoms seen in yard-long beans and weeds, which are then linked to the concentration of DNA produced. A high concentration of sample DNA can result in thick target DNA fragments (Haris et al., 2003). Although the DNA fragments produced were thin, the fact that *A*. *indica* weed was successfully amplified proved that MYMV had infected. These findings suggest that *A*. *indica* could be a source of inoculum as well as an alternative host for the spread of MYMV in yard long beans and other cultivated plants that are hosts of the virus. According to [12], *A*. *indica* is infected with Begomovirus species, allowing it to act as an alternative host.

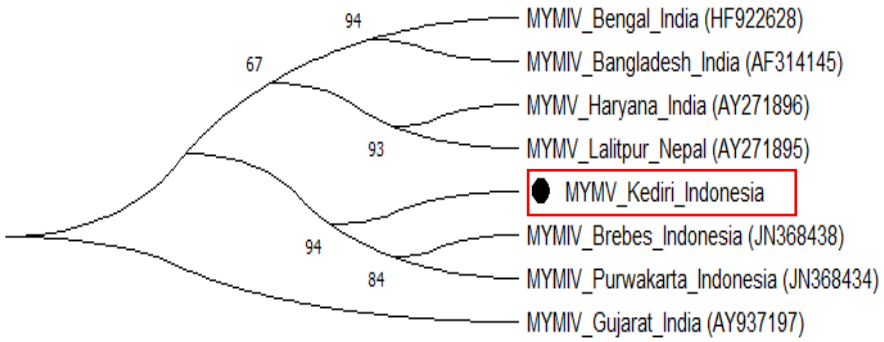

Figure 4. Phylogenetic relationships of MYMV from yard-long beans in Kencong with several isolates in GeneBank. Each node shows a bootstrap value

Figure 4 depicts the presence of one outgroup and one ingroup group in the phylogenetic tree of MYMV species. MYMIV from Gujarat, which is still a Begomovirus species, was used in the outgroup. Making a phylogenetic tree is critical for certain species that are outgroups. According to Muzzazinah, 2017), determining the starting point in the formation of a phylogenetic tree requires the use of out-group species because the derivative and primitive characteristics of the in-group species will be known later. The group is divided into two clusters, the first containing four begomovirus species and the second containing three begomovirus species (Figure 4).

An analysis of genetic diversity between MYMV isolates from yard-long beans in Kencong and other isolates at GeneBank revealed homology levels ranging from 98 to 99%. According to the phylogenetic tree (Figure 4), the MYMV isolate from Kencong had the closest relationship *with Mungbean yellow mosaic India virus* (MYMIV) isolates from soybeans from Brebes and cowpea from Purwakarta. A bootstrap value of 94 in 1000 replicates demonstrated the homology between isolates, which was included in one cluster. The phylogenetic tree reveals two clusters. The first homology included Indonesian MYMV isolates from Kediri, Brebes, and Purwakarta, while the second included MYMIV isolates from India and Nepal. The cluster classification occurred in the sample isolates as well (Nurulita et al., 2015) MYMV isolates from Indonesia differed from isolates from India, Nepal, and Pakistan in clusters.

Conclusion

Typical symptoms of yard-long beans associated MYMV i.e., mosaic with a yellow-green pattern, yellow mosaic, yellowing, leaf malformation, and stunting. Mosaic symptoms are present in several *A*. *indica*. The CTAB method was used to isolate total DNA, yielding a concentration of ±100 ng with λ DNA as a comparison. Specific primers (AC2-F & AC2-R) have successfully amplified MYMV in yard-long beans and *A*. *indica*. Phylogenetic analysis revealed that the MYMV

from Kencong was most closely related to the *Mungbean yellow mosaic India virus* (MYMIV) from soybean-Brebes and cowpea-Purwakarta.

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