KOMUNIKASI SINGKAT

Development of Specific Detection for Mungbean Yellow Mosaic India Virus Infecting Yard Long Bean in Java, Indonesia

Pengembangan Deteksi Spesifik untuk Mungbean Yellow Mosaic India Virus yang Menginfeksi Kacang Panjang di Jawa, Indonesia

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ABSTRACT

Yellow mosaic disease was reported for the first time in Indonesia in 2008. Its infection on yard long bean caused significant yield loss. Mungbean yellow mosaic India virus (MYMIV), member of genus Begomovirus was identified as the main causal agent. Specific and accurate detection is important for disease monitoring as part of disease management strategy. The aim of this study was to construct specific primer pairs for quick and robust detection of MYMIV using polymerase chain reaction method. A pair of primers MY1/MY2 was designed in this study to amplify part of MYMIV coat protein. In silico and in vitro test showed that MY1/MY2 primers specifically amplified MYMIV.

Key words: begomovirus, coat protein, MYMIV, polymerase chain reaction

ABSTRAK

Penyakit mosaik kuning pertama kali dilaporkan di Indonesia pada tahun 2008. Infeksi penyakit ini pada tanaman kacang panjang mengakibatkan kehilangan hasil yang nyata. Mungbean yellow mosaic India virus (MYMIV), anggota dari genus Begomovirus, diidentifikasi sebagai penyebab utama penyakit mosaik kuning tersebut. Deteksi yang spesifik dan akurat sangat diperlukan untuk pemantauan penyakit sebagai bagian dari strategi pengendalian penyakit. Penelitian ini bertujuan merancang primer spesifik untuk deteksi cepat MYMIV menggunakan metode polymerase chain reaction. Primer MY1/MY2 dirancang untuk mengamplifikasi sebagian protein selubung dari MYMIV. Pengujian secara in silico dan in vitro menunjukkan bahwa primer spesifik MY1/MY2 mampu mengamplifikasi secara spesifik MYMIV.

Kata kunci: begomovirus, MYMIV, polymerase chain reaction, protein selubung

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Outbreak of yellow mosaic disease on yard long bean plants in Java was first reported in 2008 (Damayanti et al. 2009). Further detection and identification confirmed that Mungbean yellow mosaic India virus (MYMIV), member of genus Begomovirus as the main causal agent of the disease (Tsai et al. 2013; Nurulita et al. 2015). Phylogenetic analysis showed that MYMIV isolate from Java was grouping together with other MYMIV isolates from South Asia and separated from the other species of the genus member of Begomovirus from Indonesia (Nurulita et al. 2015). This phylogenetic analysis was based on top region sequences which covers the unique sequence character of begomovirus including TATA box, repetitive sequence, and hairpin loop structure (Lazarowitz 1987; Usharani et al. 2004; Nurulita et al. 2015).

Detection of begomovirus in Indonesia was commonly conducted by polymerase chain reaction (PCR) method using degenerate primer. Several degenerate primers for regular begomovirus detection are PAL1v 1978/ PAR1c 715 which amplifies top region and part of coat protein (Rojas et al. 1993); Deng A/Deng B which covers partial coat protein of DNA-A (Deng et al. 1994); AV 414/AC 1048 which detects coat protein (Wyatt and Brown 1996); and currently SPG1/SPG2 (Li et al. 2004) became popular on current begomovirus studies (Kintasari et al. 2015; Wiratama et al. 2015; Listihanini et al. 2019; Pangesti et al. 2022; Selangga and Listihanini 2022). Although these degenerate primers work well for detection of many begomoviruses, specific primer is needed for the development of reliable and robust detection especially for disease monitoring in the field. This is especially important to control the spread of the disease.

Since it was first reported in 2008, the incidence of yellow mosaic disease on yard long bean has always been found in the field. Recently, in 2022 yellow mosaic disease has been found on yard long bean fields in Bogor which had similar symptoms as previously described by Tsai et al. (2013) and Nurulita et al. (2015) (Figure 1). In order to monitor the spread of yellow mosaic disease of yard long bean using routine detection assay, it is necessary to develop specific detection method for MYMIV. Therefore, the aim of this study is to design specific primer for detection of MYMIV, especially for Indonesian isolates.

A specific primer pair was constructed using in silico and confirmed through in vivo experiments. The primer was designed using primer 3 and BLAST program (as known as Primer BLAS) on NCBI (https://www.ncbi.nlm.nih.gov/tools/primer-blast/). This method was done by submitting the target sequences that obtained from pTgl isolate (Nurulita et al. 2015) to primer BLAST program at NCBI and automatically the program will select several possible primer pairs (Figure 2). The selected primers then evaluated for their properties in

Figure 1 Yellow mosaic disease on yard long bean in Bogor after one decade. a, 2012 and b, 2022.
oligonucleotide calculator including BLAST to check their specificity ([http://biotools.nubic.northwestern.edu/OligoCalc.html](http://biotools.nubic.northwestern.edu/OligoCalc.html)) (Figure 3). One forward and one reverse primers were successfully constructed, i.e. MY1 (5'-TTACATGGTCCCTCGCAACC-3') and MY2 (5'-ACAGCCTTCTCTACCCCGAT-3'), respectively. These specific primers is expected to amplify ±238 bp DNA fragment of MYMIV which covers short part of coat protein region.

Validation for specificity of these primers was conducted in vivo by PCR. Specificity of these two primers was tested using several leaf samples (Table 1). Total DNA extraction was isolated from fresh infected leaves by CTAB method (Doyle and Doyle 1987) with minor modification on omitting of phenol. Amplification was done as described earlier (Nurulita et al. 2015) with modification on annealing condition at 61 °C. This primer pair, MY1/ MY2, has 55% GC content which explained their higher annealing temperature than degenerate primer PAL1v1978/PAR1c715 that has annealing temperature of 50 °C. DNA fragment was successfully

Figure 2  Construction of specific primer using: a, primer BLAST program at NCBI and b, evaluation of its combability in OligoCalc. i, forward primer and ii, reverse primer.
amplified from all yard long bean samples using MY1/MY2, but no amplification was obtained for yard long bean, chilli pepper, cucumber, eggplant, and rice plant samples (Figure 3). These results indicated that MY1/MY2 can be used to distinguish MYMIV from other begomoviruses.

The use of specific primer for detection of MYMIV has been reported in previous studies. Primer pairs AC2-F/AC2-R, AC3-F/AC3-R, and AC4-F/AC4-R were designed to amplify MYMIV from mungbean, urdbean, and pigeon pea samples in India, respectively (Mishra et al. 2010); DNA-A forward/DNA-A reverse and DNA-B forward/DNA-B reverse were used to detect full-length of DNA-A of MYMIV from Phaseolus vulgaris sample in Nepal (Shahid et al. 2012). All these studies indicated diversity of MYMIV from different region in the world, including Indonesia. These primers MY1/MY2 can be used as early diagnosis tools, especially to anticipate reemergence disease outbreak.

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REFERENCES


