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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

Sari Nurulita, Kikin Hamzah Mutaqin, Sri Hendrastuti Hidayat*
IPB University, Bogor 16680

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 dilaporkam di Indonesia pada tahun 2018. 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

*Coressponding author: Departemen Proteksi Tanaman, Fakultas Pertanian, Institut Pertanian Bogor. Jalan Kamper, Kampus IPB Dramaga, Bogor, Jawa Barat 16680.
Phone: +62251-8629364, Fax: +62251-8629362, Email: srihendrastuti@apps.ipb.ac.id

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; Listihani *et al.* 2019; Pangesti *et al.* 2022; Selangga and Listihani 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.

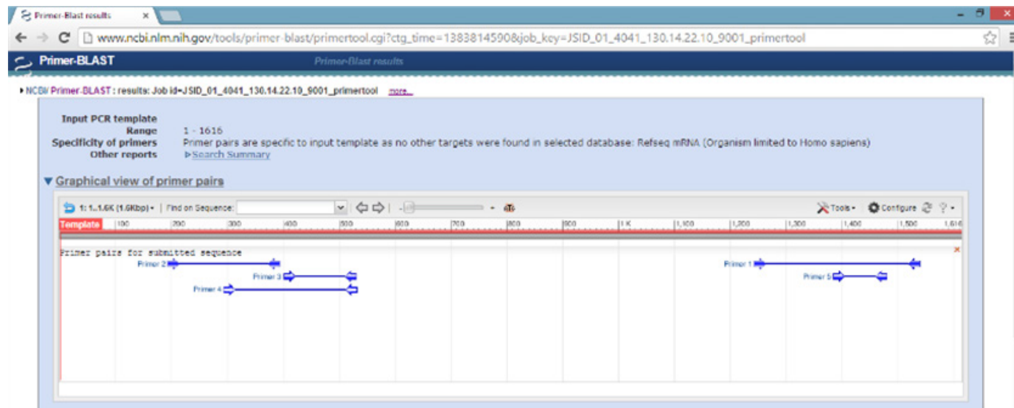
Since it was first reported in 2008, the incidence of yellow mosaic disease on yard long bean has always been found in the field. Recently, in 2022 yellow mosaic disease has been found on yard long bean fields in Bogor which had similar symptoms as previously described by Tsai *et al.* (2013) and Nurulita *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

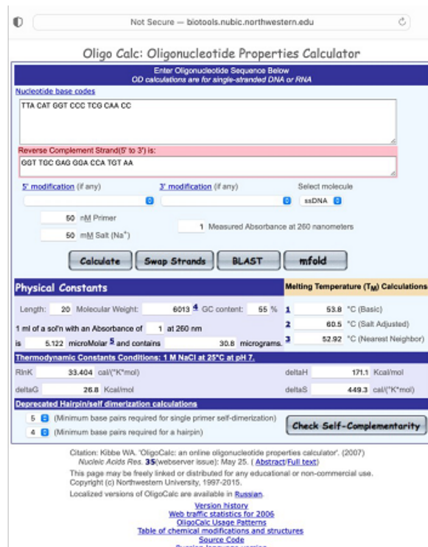
b

Figure 1 Yellow mosaic disease on yard long bean in Bogor after one decade. a, 2012 and b, 2022.

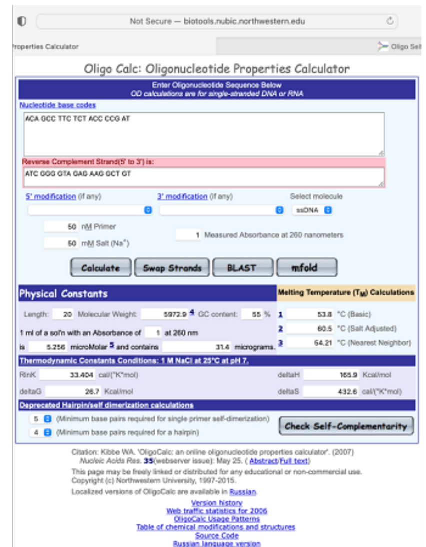
A specific primer pair was constructed using *in silico* and confirmed through *in vivo* experiments. The primer was designed using primer 3 and BLAST program (as known as Primer BLAS) on NCBI (https://www.ncbi.nlm.nih.gov/tools/primer-blast/). This method was done by submitting the target sequences 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



a



i



ii

b

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.

oligonucleotide calculator including BLAST to check their specificity (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

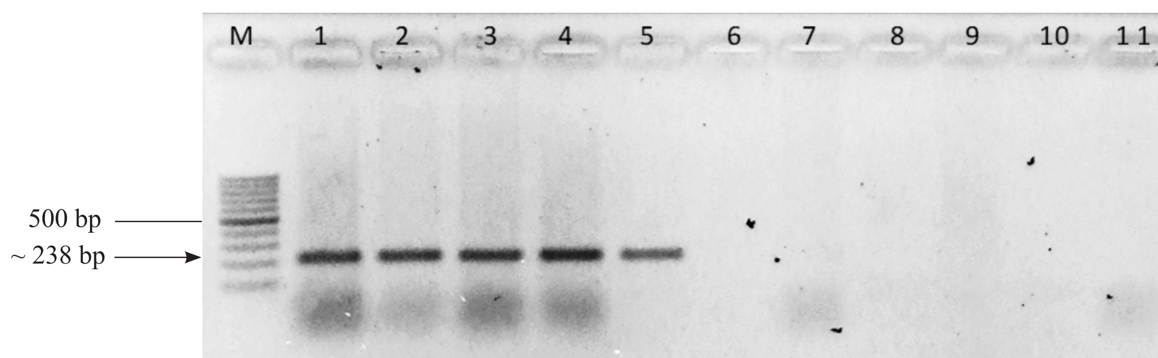


Figure 3 Amplification of partial of *Begomoviruses* using MY1/MY2 primer. M, DNA marker 100 bp (Thermo-Fisher Scientific, Waltham, US); 1, MYMIV Klaten; 2, MYMIV Magelang; 3, MYMIV Tegal; 4, MYMIV Subang; 5, MYMIV Bogor; 6, TYLCKaV from eggplant; 7, ToLCNDV from cucumber; 8, PepYLCIV from chili pepper; 9, BCMV from yard long bean; 10, RTBV from rice plant; 11, negative control.

Table 1 Virus isolates obtained for specificity assay of MYMIV primers

Virus isolates	Host	Collection/ Source
Bean common mosaic virus (BCMV)	Yard long bean	Melinda *et al*. (2016)
Mungbean yellow mosaic India virus (MYMIV)	Yard long bean	Nurulita *et al*. (2015)
Pepper yellow leaf curl Indonesia virus (PepYLCIV)	Chilli pepper	Plant Virology Laboratory, Department of Plant Protection IPB University
Tomato leaf curl New Delhi virus (ToLCNDV)	Cucumber	Septariani *et al*. (2014)
Tomato yellow leaf curl Kanchanaburi virus (TYLCKaV)	Eggplant	Kintasari *et al*. (2014)
Tungro virus	Rice plant	Ladja *et al*. (2016)

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 MYMIV on several legumes in Pakistan (Ilyas *et al*. 2010); NM1/ NM2 and MYMIV-MPF/ MYMIV-MPR were designed to amplify DNA-A and DNA-B of MYMIV, respectively, from *Vigna hainiana* and *V. trilobata* in India (Naimuddin *et al*. 2011); and HOG1/ HOG2 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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