MOLECULAR CHARACTERIZATION OF *Begomovirus* INFECTING YARD LONG BEAN (*Vigna unguiculata* subsp. *sesquipedalis* L.) IN JAVA, INDONESIA

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ABSTRACT

Begomovirus was identified as one of the causal agents associated with yellow mosaic disease on yard long bean (Vigna unguiculata subsp. sesquipedalis L.) in Java. Previous study reported that Begomovirus has infected several Leguminosae in South Asia. Several Begomoviruses have been reported to infect important crops in Indonesia. Those Begomoviruses were characterized based on nucleotide sequences. This study was conducted to identify and characterize Begomovirus taken from yard long bean samples in Java based on specific genome property of common region. Three main activities were conducted: i) sample collection in yard long bean fields located in Central Java (Tegal, Magelang, and Klaten), Yogyakarta (Sleman) and West Java (Bogor and Subang) provinces; ii) virus detection using I-ELISA, PCR and sequencing; iii) molecular characterization of *Begomovirus* using software BioEdit v.7.0.5 and MEGA 6.06. Yellow mosaic disease was found in almost all fields. Infection of Potyvirus and Begomovirus was detected using I-ELISA and PCR, respectively. Both viruses were detected as either single or mixed infection. Samples collected from Tegal, Klaten, Magelang, Subang and Bogor were positively infected by Begomovirus based on specific viral DNA amplification. Sequence analysis indicated that Begomovirus infecting yard long bean is Mungbean Yellow Mosaic India Virus (MYMIV) and it belongs to the same group with MYMIV from Bangladesh, India, Pakistan and Nepal. Further analysis showed the conserved region of Begomovirus around Common Region, i.e. "TATA box" sequence, hair pin loop structure, repetitive sequence and the conserved nonanucleotide sequence TAATATTAC were also determined. This is the first report of MYMIV infection in Indonesia.

Keywords: Begomovirus, common region, DNA sequencing, I-ELISA, MYMIV, PCR, yard long bean

INTRODUCTION

A yellow mosaic disease outbreak was reported in yard long bean (*Vigna unguiculata* subsp. *sesquipedalis* L.) growing area in Java since 2008 (Damayanti *et al.* 2009). The disease was associated with infection of Bean Common Mosaic Virus (BCMV), Cucumber Mosaic Virus (CMV) and *Begomovirus* (Damayanti *et al.* 2009; Hidayat 2011, *personal communication*; Tsai *et al.* 2013). At the same time, similar yellow mosaic disease caused by *Begomovirus* was first reported from Pakistan (Ilyas *et al.* 2010) and Nepal (Shahid *et al.* 2012).

Begomovirus is a member of *Geminivirus* group which is transmitted in nature by whitefly *Bemisia tabaci* Gen. (Hemiptera: Aleyrodidae). Molecular

characteristics of its genome is very unique, having one or two circular single stranded DNA (2.6–2.7 kb) and represented as monopartite or bipartite, respectively (Hull 2002). Serious crop diseases caused by infection of Begomovirus have been reported in Indonesia. Pepper Yellow Leaf Curl Virus (PepYLCV), Tomato Yellow Leaf Curl Virus (TYLCV), and Tobacco Leaf Curl Virus (TLCV) are considered as the major factor causing yield loss on chilli pepper, tomato and tobacco, respectively (Aidawati et al. 2005; Hidayat et al. 2006; 2008). Damage and potential yield loss in yard long bean caused by Begomovirus should be anticipated by establishing detection method and disease control strategy. To do so, basic information regarding molecular and biological characters of the virus is required.

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Method for detection of Begomovirus is commonly based on polymerase chain reaction (PCR) technique using universal primers. Degenarate primers PAL1v1978/ PAR1c715 was first used by Rojas et al. (1993) as universal primer to detect several Geminiviruses from infected plants belong to Solanaceae, Leguminosae, Euphorbiaceae and Malvaceae from South America. Since then, the primer pair has been used to identify and characterize various members of Begomovirus. Fragment of Begomovirus DNA amplified by this pair of primers covers part of AL1 region (replicase gene), common region and intergenic region and part of AR1 region (coat protein gene). Molecular characters of Begomovirus can be determined by analyzing this DNA fragment sequences due to its conserved and diversed region. Common region for each Begomovirus is different, except loop region on hairpin structure, 5'-TAATATTAC-3' which is known as the conserved nonanucleotide region (Lazarowitz 1987). *Begomovirus* possessing >92% similarity on their hairpin loop structure can be grouped as one species (Fauquet et al. 2005).

This paper explained the attempts to identify and characterize *Begomovirus* from yard long bean samples in Java based on specific genome property of common region.

MATERIALS AND METHODS

Collection of Field Samples

Samples collection was conducted in West Java (Bogor and Subang), Central Java (Tegal, Klaten and Magelang) and Yogyakarta (Sleman). Leaf samples were collected from the plants age 6–9 WAP using purposive sampling method based on yellow mosaic symptom described by Damayanti *et al.* (2009) and Ilyas *et al.* (2010). Fresh tissue was directly subjected for virus detection and the remaining were stored as isolate collection in the laboratory at -80 °C.

Virus Detection by Indirect Enzyme-linked Immunosorbent Assay (I-ELISA)

Two major viruses on yard long bean i.e. *Potyvirus* and CMV were detected from leaf samples using I-ELISA commercial kit following manufacturer protocol (Leibniz-Institut DSMZ GmbH, Germany). Infected leaves was ground in

coating buffer (1/10, v/v). Coating buffer (pH 9.6) containing 15 mM Na₂CO₃ (1.59 g), 2.38 mM NaHCO₃ (2.93 g), 3.08 mM NaN₃ (0.20 g) and H₂O was added to final volume of 1 L. Aliguots of each sample (100 µL) was dispensed to each microtitters' well, then incubated overnight at 4 °C. Microtitter plate was washed 8 times using PBS-Tween (0.137 M NaCl (8.0 g), 1.47 mM KH₂PO₄ (0.2 g), 8.1 mM Na₂HPO₄ (1.15 g), 2.68 mM KCI (0.2 g), 3.08 mM NaN₃ (0.2 g), add H₂O up to 1 L pH 7.4 and add 0.5 ml Tween 20/ L). Plates were dried up by tapping upside down on tissue paper. Blocking solution (2% skim milk diluted in PBS-Tween) was added 100 µL to each well then incubated at 37 °C for 30 minutes. Blocking solution was removed and plate was tapped dry. First antibody (IgG) was diluted in conjugate buffer (PBS-Tween containing 2% PVP (Serva PVP-15 polyvinyl pyrrolidon) and 0.2% egg albumin) according to manufacturer recommendation, then 100 µL conjugate buffer contains IgG was added to each well, incubated at 37 °C for 2-4 hours, then washed by PBS-Tween. Second antibody (IgG-AP) was diluted in conjugate buffer according to manufacturer recommendation, then 100 µL conjugate buffer containing IgG-AP was added to each well, incubated at 37 °C for 2 hours then washed using PBS-Tween. Substrate (10 mg p-nitrophenyl phosphate) was dissolved in substrate buffer (97 mL diethanolamine, 600 mL H₂O, 3.08 mM NaN₂ (0.2 g), adjust to pH 9.8 with HCl and make up to 1 L with H₂O) added 100 µL to each well. Plate was incubated in the room temperature under low light intensity and reaction was evaluated. Positive reaction was qualitatively indicated by appearance of yellow color and quantitatively determined by measuring absorbance value at 405 nm wave length, i.e. two times absorbance value of negative control (healthy plant), using ELISA reader (Biorad 550).

Virus Detection by PCR

Total viral DNA was isolated from infected leaf following a procedure described by Doyle and Doyle (1987) with minor modification. Fresh tissue (0.1 g) was ground with liquid nitrogen to powder, 500 μ L of CTAB buffer (10% Cetyltrimethyl-ammonium bromide (100 mL), 0.1 M Tris-HCl pH 8 (100 mL), 0.05 M EDTA (50 mL), 0.5 M NaCl (126 mL), 1% β-mercapto-ethanol

(10 mL and added H₂O up to 1 L) was added and the sap was transferred to 1.5 mL clean tube. The sap was incubated in water bath at 65 °C for 1 hour, shaked every 10 minutes to separate lipid and protein. Five hundred microliter of chloroform/iso-amyl alcohol (24:1, v/v) was added to the liquid, then the tube was vortexed for 5 minutes and centrifuged at 14,000 rpm for 15 minutes. The supernatant was pipetted to 1.5 mL clean tube, 1/10 volume of 3 M ammonium acetate and 2/3 volume of isopropanol was added, respectively. The liquid was mixed gently then incubated overnight at -20 °C or 4 hours at room temperature. After incubation, the liquid was centrifuged at 12,000 rpm for 10 minutes to precipitate DNA and then was discarded flowthrough. The pellets were washed with 500 µL of 70% ethanol, centrifuged at 8,000 rpm for 5 minutes and dried in room temperature after discarding the flow through. The dried pellets containing total DNA were dissolved in 50 to 100 µL of nuclease free water or TE buffer, pH 8 and the DNA was ready for PCR.

Amplification of viral DNA was conducted following method described by Rojas et al. (1993) to confirm geminivirus infection. PCR reaction contained 10xPCR Buffer, 25mM MgCl₂, 2.5mM dNTPS, 10µM each of primer PAL1v1978 and PAR1c715, Taq polymerase (5U/µL), 1 µL of DNA and the reaction was adjusted to 25 µL with nuclease free water. Amplifications was performed in GeneAmp PCR System 9700 machine with 5 minutes at 94 °C for pre-heating, followed by 30 cycles of denaturation (1 minute at 94 °C), annealing (1 minute at 50 °C) and extension (3 minutes at 72°C). The last cycle was followed by 72 °C for 3 minutes and decreased at 4 °C. Agarose gel electrophoresis was used to visualize PCR products.

DNA Sequencing

Viral DNA fragments obtained from direct PCR amplification were sent to PT Genetika Science, Indonesia and Australian Genome Research Facility, Australia, respectively for DNA sequencing. Sequence data were compared with other sequences from GenBank (NCBI 2013) and analysed using software programs BioEdit V.7.0.5, CLC Sequence Viewer 7, and MEGA 6.06.

RESULTS AND DISCUSSION

Identification of *Begomovirus* from Field Samples

Incidence of yellow mosaic disease was very high i.e. 80% to 100% in most growing areas. Infected plants were easily recognized in the field based on visual symptoms. Three main symptoms of yellow mosaic disease in the field were observed i.e. 1) yellowing; 2) yellowing with green spot; and 3) mosaic vein banding (Fig. 1 and Table 1). The most common symptom found in every field was yellowing. Yellowing with green spot was thought as early symptom before it developed into yellowing. Further severe infection caused smaller pods and leaves. These types of symptom had also been reported as typical symptoms of yellow mosaic disease of yard long bean in South Asia (Ilyas et al. 2010). Begomovirus was detected in 11 infected plant samples (Fig. 2) showing yellowing and yellowing with green spot symptoms from all locations. However, Begomovirus fragment was not successfully amplified from samples showing mosaic vein banding from Subang Viral detection using I-ELISA revealed the infection of Potyvirus



Figure 1. Symptoms of yellow mosaic disease on yard long bean: (A) yellowing; (B) yellowing with green spot; (C) mosaic vein banding

Location	Code of looksta		I-E	PCR	
Location Code of Isola	Code of Isolates	Symptoms description	CMV	Potyvirus	Begomovirus
Tegal	Tegal 1	Yellowing	-	+	+
	Tegal 2	Yellowing mosaic	-	-	+
Klaten	Klaten 1	Yellowing	-	-	+
	Klaten 2	Yellowing mosaic	-	-	-
Sleman	Sleman 1	Yellowing	-	+	+
	Sleman2	Yellowing mosaic	-	+	+
Magelang	Magelang 1	Yellowing mosaic	-	-	+
	Magelang 2	Yellowing	-	+	+
	Magelang 3	Yellowing mosaic	-	-	+
Subang	Subang 1	Mosaic vein banding	-	+	-
	Subang 2	Mosaic vein banding	-	+	-
	Subang 3	Mosaic vein banding	-	-	-
	Subang 4	Yellowing	-	-	+
Bogor	Bogor 1	Yellowing	-	-	+
	Bogor 2	Yellowing	-	-	+

Table 1. Detection of Cucumber Mosaic Virus, *Potyvirus* and *Begomovirus* from leaf samples collected in wet season 2012 from various locations using I-ELISA and PCR^a

^a Most plants were in generative stage when samples was collected

^b - = not detected; + = detected



Figure 2. Visualization of *Begomovirus* amplification from leaf samples using universal primers PAL1v1978/ PAR1c715 on 1% agarose gel. M, 1 kb marker DNA (Thermo Scientific, US); K+, DNA of PepYLCIV; 1-15 leaf samples from fields (1, Tegal 1; 2, Tegal 2; 3, Klaten 1; 4, Klaten 2; 5, Sleman 1; 6, Sleman 2; 7, Magelang 1; 8, Magelang 2; 9, Magelang 3; 10, Subang 1; 11, Subang 2; 12, Subang 3; 13, Subang 4; 14, Bogor 1; 15, Bogor 2)

but not of Cucumber Mosaic Virus (CMV). Mosaic vein banding symptom caused by *Potyvirus* infection had been described previously by Damayanti *et al.* (2009). This result indicated the association of *Begomovirus* with yellow mosaic disease of yard long bean in Java.

Analysis of Sequence Identity of *Begomovirus* Infecting Yard Long Bean

Nucleotide sequences were obtained for *Begomovirus* isolates from Tegal, Klaten, Magelang, Subang and Bogor. The sequence of isolate from Sleman was not good due to unreadable

chromatogram, therefore it was not included in the further sequence analysis. Analysis of their identity by comparing to sequences on the GeneBank showed their highest homology with Mungbean Yellow Mosaic India Virus (MYMIV) from Brebes and Purwakarta, i.e. >92%, followed by MYMIV from Bangladesh, Nepal, Pakistan and India, i.e. >87% (Table 2). Their homology to MYMV, another virus causing yellow mosaic disease in South Asia, was only 71-76% and to other *Begomovirus* reported from Indonesia was even lower i.e. 51- 54%. Further dendogram analysis to study their relationship showed that all

Begomovirus infecting		Begomovirus isolates from GeneBank ^a)										
yard long bean	1	2	3	4	5	6	7	8	9	10	11	12
pTgl1	99.7	97.9	92.5	91.1	92.2	92.1	76.0	55.8	53.8	51.4	51.5	16.6
Tegal 2	95.7	94.7	89.5	88.3	89.4	89.3	73.1	54.6	53.9	52.5	52.0	17.4
Klaten	96.0	93.2	88.0	86.8	87.9	87.9	71.9	54.1	54.2	52.9	51.9	17.2
Magelang	95.9	92.8	87.7	86.4	87.5	87.5	71.7	53.5	53.8	52.1	51.6	16.8
Magelang 2	95.3	92.8	87.7	86.4	87.5	87.5	71.7	53.5	53.8	52.1	51.6	16.8
Subang	95.5	94.7	89.5	88.3	89.4	89.3	73.0	54.6	54.0	52.5	52.0	17.4
Bogor 1	95.4	94.8	89.6	88.4	89.5	89.4	73.0	54.7	54.1	52.5	52.2	17.4
Bogor 2	95.1	93.8	88.6	87.4	88.5	88.4	72.6	53.7	54.1	52.7	52.1	17.5

Table 2. Nucleotide sequence homology (%) of *Begomovirus* infecting yard long bean in Java with other *Begomoviruses* reported earlier in GeneBank

^{a)}1= MYMIV Brebes (JN368436); 2= MYMIV Purwakarta (JN368434); 3= MYMIV India (KC852204); 4= MYMIV Bangladesh (AF314145); 5= MYMIV Nepal (AY271895); 6= MYMIV Pakistan (AM992618); 7= MYMV India (KC911271); 8= PepYLCIV (AB246170); 9= TLCIV (AB241671), 10= ToLCNDV (data unpublished); 11= ToLCJV (189848); 12= BCMV Bogor (FJ653916)



Figure 3. Phylogenetic analysis of Mungbean Yellow Mosaic India Virus based on alignment of partial nucleotide sequences of the DNA-A of *Begomoviruses* using Mega 6.06 (Algorithm Neighbor Joining with 1,000 bootstraps replicates)

Begomovirus infecting mungbean (MYMIV and MYMV) belonged to similar cluster and they were separated from *Begomoviruses* infecting other crops (PepYLCIV, TLCIV, TYLCNDV, ToLCJV) (Fig. 3). It indicated that MYMIV from Java had closer genetic relationship to MYMIV from South Asia than other *Begomoviruses* from Indonesia.

Analysis of Common Region Sequences of Begomovirus Infecting Yard Long Bean

Molecular characters of *Begomovirus* could be determined by analyzing the top region of its genome covering common region and intergenic region representing the conserved and diversed region, respectively. Common region for each *Begomovirus* was different, except loop region on

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	1 1				
	5	15	25	 35	45
MYMIV_pTgl	TTT-GAAGTC	GTTTTTGTAT		CCGATTGC-T	TCTCTACCCC
MYMIV_Tegal				CCGATTGC-T	
MYMIV_Klaten				CCGATTGC-T	
MYMIV_Magelang1 MYMIV_Magelang2				CCGATTGC-T CCGATTGC-T	
MYMIV_Nagerang2 MYMIV_Subang				CCGATTGC-T	
MYMIV Bogorl				CCGATTGC-T	
MYMIV Bogor2				CCGATTGC-T	
MYMIV_Brebes				CCGATTGC-T	
MYMIV_Purwakarta				CCGATTGC-T	
MYMIV_India				CCGATTGCCT	
MYMIV_Bangladesh MYMIV Nepal				CCGATTGC-T CCGATTAC-T	
MYMIV Pakistan				CCGATTAC-T	
MYMV India				CTAAAAGTCC	
PepYLCIV	TGG-AGTGTC	GTTTTGTATT	GGAGACAATC	ACTTCTATCC	CTATGTATTG
TLCIV				AAACTTGCGT	
TolCNDV				CAACTTCCTC	
ToLCJV BCMV Bogor				ATAGTT-CTC ACCAATATCA	
BCHV_BOGOT	16A6661A	AACCACACIT	1611611600	ACCAATATCA	ICGAGAACGG
	55	65	75	85	95
MYMIV_pTgl				TATACTAGAG	
MYMIV_Tegal				TATACTAGAG	
MYMIV_Klaten MYMIV Magelang1				TATACTAGAG	
MYMIV_Magelang2				TATACTAGAG	
MYMIV Subang				TATACTAGAG	
MYMIV_Bogor1	CCTATCGGT-	GTATCG	GTGTACTATA	. TATACTAGAG	CTATTAAAAG
MYMIV_Bogor2	CCTATCGGT-	GTATCG	GTGTAC TATA	. TATACTAGAG	CTATTAAAAG
MYMIV_Brebes				TATACTAGAG	
MYMIV_Purwakarta MYMIV India				TATACTAGAG TATACTAGAG	
MYMIV_Bangladesh				TATACTAGAG	
MYMIV Nepal				TATACTAGAA	
MYMIV_Pakistan	CCTATCGGT-	GTATCG	GTGTCT TATA	. TATA CTAGAG	CTACTAAAAG
MYMV_India				. TGTAGGAGAG	
PepYLCIV				TATAATGGCT	
TLCIV TolCNDV				CTAAATGGCA CTAAACGGCA	
ToLCJV				CCAAATGGCA	
BCMV_Bogor	CGT				
MYMIV_pTgl	105 CCCATA <mark>GGGG</mark>	115 CACTCAG-CT	125 <mark>А</mark> ТАА-ТАТТА	 135 CCTGAGTGCC	145 CCGCGACCGG
MYMIV_Tegal	105 CCCATA <mark>GGGG</mark> CCCATA <mark>GGGG</mark>	115 CACTCAG-CT CACTCAG-CT	125 ATAA-TATTA ATAA-TATTA	135 CCTGAGTGCC CCTGAGTGCC	145 CCGCGACCGG CCGCGACCGG
	105 CCCATA <mark>GGGG</mark> CCCATA <mark>GGGG</mark> CCCATA <mark>GGGG</mark>	115 CACTCAG-CT CACTCAG-CT CACTCAG-CT	125 ATAA-TATTA ATAA-TATTA ATAA-TATTA	135 CCTGAGTGCC CCTGAGTGCC	145 CCGCGACCGG CCGCGACCGG CCGCGACCGG
MYMIV_Tegal MYMIV_Klaten MYMIV_Magelang1 MYMIV_Magelang2	105 CCCATA <mark>GGGG</mark> CCCATA <mark>GGGG</mark> CCCATA <mark>GGGG</mark> CCCATA <mark>GGGG</mark> CCCATA <mark>GGGG</mark>	115 CACTCAG-CT CACTCAG-CT CACTCAG-CT CACTCAG-CT	125 ATAA-TATTA ATAA-TATTA ATAA-TATTA ATAA-TATTA ATAA-TATTA	135 CCTGAGTGCC CCTGAGTGCC CCTGAGTGCC CCTGAGTGCC CCTGAGTGCC	145 CCGCGACCGG CCGCGACCGG CCGCGACCGG CCGCGACCGG CCGCGACCGG
MYMIV_Tegal MYMIV_Klaten MYMIV_Magelang1 MYMIV_Magelang2 MYMIV_Subang	105 CCCATA <mark>GGGG</mark> CCCATA <mark>GGGG</mark> CCCATA <mark>GGGG</mark> CCCATA <mark>GGGG</mark> CCCATA <mark>GGGG</mark>	115 CACTCAG-CT CACTCAG-CT CACTCAG-CT CACTCAG-CT CACTCAG-CT	125 ATAA-TATTA ATAA-TATTA ATAA-TATTA ATAA-TATTA ATAA-TATTA ATAA-TATTA	135 CCTGAGTGCC CCTGAGTGCC CCTGAGTGCC CCTGAGTGCC CCTGAGTGCC	145 CCGCGACCGG CCGCGACCGG CCGCGACCGG CCGCGACCGG CCGCGACCGG CCGCGACCGG
MYMIV_Tegal MYMIV_Klaten MYMIV_Magelang1 MYMIV_Magelang2 MYMIV_Subang MYMIV_Bogor	105 CCCATA <u>GGGG</u> CCCATA <u>GGGG</u> CCCATA <u>GGGG</u> CCCATA <u>GGGG</u> CCCATA <u>GGGG</u> CCCATA <u>GGGG</u>	115 CACTCAG-CT CACTCAG-CT CACTCAG-CT CACTCAG-CT CACTCAG-CT CACTCAG-CT	125 ATAA-TATTA ATAA-TATTA ATAA-TATTA ATAA-TATTA ATAA-TATTA ATAA-TATTA ATAA-TATTA	135 CCTGAGTGCC CCTGAGTGCC CCTGAGTGCC CCTGAGTGCC CCTGAGTGCC CCTGAGTGCC	145 CCGCGACCGG CCGCGACCGG CCGCGACCGG CCGCGACCGG CCGCGACCGG CCGCGACCGG CCGCGACCGG
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MYMIV_Tegal MYMIV_Klaten MYMIV_Magelang1 MYMIV_Magelang2 MYMIV_Subang MYMIV_Bogor MYMIV_Bogor MYMIV_Bogor MYMIV_Brebes	105 CCCATA <u>GGGG</u> CCCATA <u>GGGG</u> CCCATA <u>GGGG</u> CCCATA <u>GGGG</u> CCCATA <u>GGGG</u> CCCATA <u>GGGG</u>	115 CACTCAG-CT CACTCAG-CT CACTCAG-CT CACTCAG-CT CACTCAG-CT CACTCAG-CT CACTCAG-CT CACTCAG-CT	125 ATAA-TATTA ATAA-TATTA ATAA-TATTA ATAA-TATTA ATAA-TATTA ATAA-TATTA ATAA-TATTA ATAA-TATTA	135 CCTGAGTGCC CCTGAGTGCC CCTGAGTGCC CCTGAGTGCC CCTGAGTGCC CCTGAGTGCC CCTGAGTGCC	145 CCCCCACCGG CCCCACCGG CCCCCACCGG CCCCCACCGG CCCCCACCGG CCCCCACCGG CCCCCACCGG CCCCCACCGG CCCCCACCGG
MYMIV_Tegal MYMIV_Klaten MYMIV_Magelang1 MYMIV_Subang MYMIV_Bogor MYMIV_Bogor MYMIV_Bogor MYMIV_Brebes MYMIV_Purwakarta MYMIV_India	105 CCCATAGGGC CCCATAGGGC CCCATAGGGC CCCATAGGGC CCCATAGGGC CCCATAGGGC CCCATAGGGC CCCATAGGGC CCCATAGGGC CCCATAGGGC	115 CACTCAG-CT CACTCAG-CT CACTCAG-CT CACTCAG-CT CACTCAG-CT CACTCAG-CT CACTCAG-CT CACTCAG-CT CACTCAG-CT CACTCAG-CT	125 ATAA-TATTA ATAA-TATTA ATAA-TATTA ATAA-TATTA ATAA-TATTA ATAA-TATTA ATAA-TATTA ATAA-TATTA ATAA-TATTA ATAA-TATTA	135 CCTGAGTGCC CCTGAGTGCC CCTGAGTGCC CCTGAGTGCC CCTGAGTGCC CCTGAGTGCC CCTGAGTGCC CCTGAGTGCC CCTGAGTGCC	145 CCGCCACCGG CCGCCACCGG CCGCCACCGG CCGCCACCGG CCGCCACCGG CCGCCACCGG CCGCCACCGG CCGCCACCGG CCGCCACCGG CCGCCACCGG CCGCCACCGG CCGCCACCGG CCGCCACCGG
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MYMIV_Tegal MYMIV_Tegal MYMIV_Magelang1 MYMIV_Magelang2 MYMIV_Bogor MYMIV_Bogor MYMIV_Bogor MYMIV_Brebes MYMIV_Brebes MYMIV_Brebes MYMIV_Purwakarta MYMIV_Bangladesh MYMIV_Bangladesh MYMIV_Pakistan MYMV_India PepYLCIV ToLCJV ToLCJV BCMV_Bogor MYMIV_Tegal MYMIV_Tegal MYMIV_Magelang1 MYMIV_Bogor1 MYMIV_Bogor1 MYMIV_Bogor2 MYMIV_Bogor2 MYMIV_Bebes MYMIV_Bebes MYMIV_Bealadesh MYMIV_Netatan MYMIV_Bealadesh MYMIV_Netatan MYMIV_Pakistan MYMIV_Pakistan MYMIV_India PepYLCIV	105 CCCATAGGG TTTGTACACC TTTGTACACC TTTGTACACA TTTAGAAAGA TTCTCATAGA TTTAGAAAGA TCTCATAGGG TGTATTGGGG TGTATTAGGG	115 CACTCAG-CT CACTCAG	125 ATAA-TATTA ATAA-TATTA ATAA-TATTA ATAA-TATTA ATAA-TATTA ATAA-TATTA ATAA-TATTA ATAA-TATTA ATAA-TATTA ATAA-TATTA ATAA-TATTA ATAA-TATTA ATAA-TATTA ATAA-TATTA ATAA-TATTA ATAA-TATTA ATAA-TATTA ATAA-TATTA CONTINUES CONT	135 CCTGAGTGCC CTTTTGGTAC TTTTTGGTAC TTTTTGGTAC TTTTGGTAC	145 CCGCCACCGG CCTTATCTTT
MYMIV_Tegal MYMIV_Klaten MYMIV_Magelang1 MYMIV_Magelang2 MYMIV_Bogor MYMIV_Bogor MYMIV_Bogor MYMIV_Parbes MYMIV_Purwakarta MYMIV_Parbas MYMIV_Parbas MYMIV_Parbas MYMIV_Parbas MYMIV_Parbas MYMIV_Parbas MYMIV_Parbas BCMV_Bogor MYMIV_Tegal MYMIV_Tegal MYMIV_Magelang1 MYMIV_Bogor1 MYMIV_Bogor1 MYMIV_Bogor1 MYMIV_Bogor2 MYMIV_Bogor2 MYMIV_Bogor2 MYMIV_Bogor2 MYMIV_Parbas MYMIV_PArbas	105 CCCATAGGG TTGTAGGG TTGTATGGGG TGTATTGGGGG TGTATTGGGG TGTATTGGGGG TGTATTGGGGGGGG	115 CACTCAG-CT CACTCAG-CC CCGATGCC CCGATGCC CCGATGCC CCGATGCC	125 ATAA-TATTA CAAATTTG TTAATCCT CTTTAATGCT CTTTAATGCT CTTTAATGCT CTTTAATGCT CTTTAATGCT CTTTAATGCT CTTTAATGCT CTTTAATGCT CTTTAATGCT CTTTAATGCT CTTTAATGCT CTTTAATGCT CTTTAATGCT CTTTAATGCT CTTTAATGCT CTTTAATGCT CTTTTCTCGCT TTTTGCT	135 CCTGAGTGCC CTTTTGGTAC TTTTTGGTAC TTTTTGGTAC TTTTGGTAC	145 CCGCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC

Figure 4. Alignment of nucleotide sequences of the CR of Mungbean Yellow Mosaic India Virus (MVMIV) isolates from Java with other reported MYMIV in Genebank. The alignment showing repetitive sequences (grey shadow), invert repeat (green shadow), TATA sequences (bold letter with purple shadow) and the hairpin loop region (underlined letter with yellow shadow) with nonanucleotide sequence (bold and underlined letter with turquoise shadow)



Figure 5. Hairpin loop structures of several *Begomovirus* isolates. A. MYMIV Tegal; B. MYMIV Klaten; C. MYMIV Magelang; D. MYMIV Subang; E. MYMIV Purwakarta; F. MYMIV Bogor; G. MYMIV Bangladesh; H. MYMIV Pakistan; I. MYMIV India; J. MYMIV Nepal; K. TLCIV Indonesia; L. PepYLCIV Indonesia

hairpin structure, 5'-TAATATTAC-3' which is known as the conserved nonanucleotide region (Lazarowitz 1987).

Comparison of iteron and protein products variations have been used to determine of MYMV and MYMIV from several regionals in India (Varma & Malathi 2003; Usharani *et al.* 2004) and TYLCV isolates from Israel, Sardinia and Thailand (Argüelo-Astorga *et al.* 1994). However, exception was reported on several cases, for example Potato Yellow Mosaic Virus with TYLCV-Sardinia and BGMV-Brazil with TYLCV-Israel have identical iterons although they have a rather far distance relationship (Argüelo-Astorga *et al.* 1994).

Our analysis showed that common region of Begomovirus isolates infecting yard long bean in Java consists of repetitive sequences (ATCGGTGT), TATA box, and hair pin loop structure (Fig. 4). Three direct repeats (two of them are tandem repeat sequences) can be found before TATA box in all MYMIV and MYMV isolates (Fig. 4). Iteron and TATA box sequence always present together in CR of each Begomovirus species (Lazarowitz 1987). The function of iteron and TATA box was described by Argüelo-Astorga et al. (1994) as initiator for rolling circle replication which was known as RAP-specific binding sites. Similar characteristics were reported for Eastern Hemisphere geminivirus except ACMV and ICMV, Western Hemisphere geminivirus, and SqLCV-E and -R (Argüelo-Astorga et al. 1994), and MYMIV on soybean (Usharani et al. 2004).

Hair pin loop structure of each isolates was identical to MYMIV from Bangladesh, Pakistan, India, Nepal, and one isolate of PepYLCIV, but they were different from TLCIV and PepYLCIV (Fig. 5). *Begomovirus* possessing >92% similarity on their hairpin loop structure could be grouped as one species (Fauquet *et al.* 2005; Hidayat *et al.* 2008). Therefore, all *Begomovirus* isolates from this study was the same species with MYMIV from Bangladesh, India, Pakistan, and Nepal.

CONCLUSIONS

Molecular detection and characterization confirmed the association of MYMIV in yellow mosaic disease of yard long bean in Java. Virus isolates from Java had the highest similarity (>85%) with MYMIV isolates from Bangladesh, Pakistan, India, and Nepal. Further study on characters of the virus, such as host range, insect transmission and disease spread, are very important to understand disease development and control.

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