Jurnal Perlindungan Tanaman Indonesia, Vol. 23, No. 2, 2019: 197–204
DOI: 10.22146/jpti.41402
Available online at http://jurnal.ugm.ac.id/jpti
ISSN 1410-1637 (print), ISSN 2548-4788 (online)

Research Article

Molecular Characterization of *Begomovirus* on Cucumber in Java

Listihani[1)], Tri Asmira Damayanti[1)]*, Sri Hendrastuti Hidayat[1)], & Suryo Wiyono[1)]

[1)]*Department of Plant Protection, Faculty of Agriculture, IPB University*
Jln. Kamper, Kampus IPB Dramaga, Bogor, West Java 16680 Indonesia

**Corresponding author. E-mail: triadys@apps.ipb.ac.id*

Received November 28, 2018; revised January 25, 2019; accepted April 15, 2019

ABSTRACT

A survey on several cucumber cultivation areas in West Java, Central Java, Yogyakarta, and East Java found many plants showing typical *Begomovirus* symptoms such as yellow mosaic, cupping, and vein banding. This study was aimed to determine virus frequency, detection and molecular characterization of the causal virus of those symptoms on cucumber in Java. Sampling was conducted by collecting 50 symptomatic plants from each location in West Java (Indramayu, Subang, and Bogor), Central Java (Brebes and Klaten), Yogyakarta (Kulon Progo), and East Java (Nganjuk, Kediri, and Tulungagung) using purposive sampling method. The virus frequency was determined serologically based on DIBA test using specific antiserum of *Tomato leaf curl New Delhi virus* (ToLCNDV) and *Squash leaf curl virus* (SLCV). The DNA detection and identification was conducted by PCR using specific primer of ToLCNDV and SLCV, DNA cloning, and sequencing. The results of serological detection showed the average frequency of ToLCNDV and SLCV ranged from 92.77–100% and 78.33–93.3%, respectively. PCR using specific primer of ToLCNDV successfully amplified the coat protein gene with sized ±600 bp from selected samples. However, SLCV not amplified on all samples by PCR using specific primer, indicating its existence might not present yet on cucumber in Java. Homology nucleotide and amino acid sequences among ToLCNDV Java isolates ranging from 95.6–99.2% and 99.7–100%. ToLCNDV isolates Java had highest nucleotide and amino acid sequences similarity with cucumber isolate from Klaten, Indonesia (AB613825) ranging from 96.1–98.1% and 99.7–100%, and was considered as "Indonesia" strain.

Keywords: Begomovirus, cucumber, Java isolates, ToLCNDV

INTRODUCTION

Cucumber (*Cucumis sativus* L.) is one of the most widely cultivated horticultural plants in Indonesia. Cucumber production in 2013–2016 tends to decrease every year from 491.636 tons, 477.976 tons, 447.677 tons, and 430.201 tons, respectively (BPS, 2017). In addition, cucumber in the fields always showed unhealthy due to infection by many types of pathogens, including viruses. Some of these viruses have been reported to infect horticultural plants in several countries, especially *Begomovirus* which caused a serious problem in cucumber (Brown *et al*., 2002).

Begomovirus is a genus member of Geminiviridae family that causes important diseases in horticultural plants, i.e. Solanaceae, Cucurbitaceae, and Malvaceae (Phaneendra *et al*., 2012; Venkataravanappa *et al*., 2012) and whitefly (*Bemisia tabaci*) is vector of Begomoviruses (Jones, 2003). *Begomovirus* infection causes curling, yellowing, and mosaic leaves in Cucurbitaceae (Ali–Shtayeh *et al*., 2014). Begomoviruses that infect the Cucurbitaceae namely, *Tomato leaf curl New Delhi Virus* (ToLCNDV), *Squash leaf curl virus* (SLCV), *Squash leaf curl China virus* (SLCCNV), *Ageratum yellow vein virus* (AYVV), and *Pepper yellow leaf curl Indonesia virus* (PYLCIV) (Bandaranayake *et al*., 2014; Wilisiani *et al*., 2014; Ali–Shtayeh *et al*., 2014). Moreover, Begomoviruses infection showed diverse symptoms such as curly leaf on melon in Bantul, Yogyakarta caused by PYLCIV, mosaic with leaf distortion symptoms on Cucurbitaceae caused by ToLCNDV (Wilisiani *et al*., 2014; Khameneh, 2016) and leaves yellowing, curl, blister, mottle, and small fruit caused by SLCV (Ali-Shtayeh *et al*., 2014). In addition, ToLCNDV is an important virus in horticultural crops in Asia (Mizutani *et al*., 2011; Pratap *et al*., 2011).

Recently, ToLCNDV have been reported to infect cucumber in West Java and Bali (Mizutani *et al*., 2011; Haerunisa *et al*., 2016). Further, ToLCNDV reported on cucumbers in Tegal, Kalasan, Ngemplak,

Sukoharjo, and Bogor with incidence up to 91.67% and showed nucleotide homology among isolates ranging from 95.7–98.6% (Septariani *et al.*, 2014). SLCCNV was reported limitedly in Apuan and Bangli Villages, Baturiti District, Tabanan Regency, Bali with incidence up to 80% (Wiratama *et al*., 2015).

We conducted field surveys on 2016 at several cucumber cultivation areas in different locations from previously reports (Septariani *et al.*, 2014; Haerunisa *et al*., 2016) to collected cucumber leaf samples. Based on field survey, we found Begomovirus-like symptoms such as yellow leaves, blister leaves, mosaics, and upward curling of the leaf edges symptoms. This research aimed to detect the virus frequency, its distribution and identification of the causal of those Begomovirus(es) symptoms.

MATERIALS AND METHODS

Survey and Sample Collection

Survey and sampling of virus-like symptom leaves from several cucumber cultivation areas were conducted in West Java Province; Bogor Regency (Situgede, Ciampea, and Tenjolaya villages), Subang Regency (Ciasem, Cikaum, and Purwadadi villages), and Indramayu Regency (Cikedung, Anjatan, and Patrol villages), in Central Java Province; Brebes Regency (Bulakamba, Wanasari, and Larangan villages), in the Special Region of Yogyakarta; Klaten Regency (Prambanan, Gantiwarno and Jogonalan villages), and Kulonprogo Regency (Temon, Panjatan, and Wates villages), in East Java Province; Nganjuk Regency (Ngronggot, Kertosono, and Prambon villages), Kediri Regency (Papar, Pagu, and Semampir villages), and Tulungagung Regency (Ngantru, Kedungwaru, and Pakel villages). Samples were collected by using a purposive sampling method to collect 50 symptomatic samples from each location. Total samples collected up to 543 samples.

Determination of Virus Frequency

Serological detection was conducted using the Dot Blot Immunobinding Assay (DIBA) using specific antiserum of ToLCNDV and SLCV (DSMZ, Germany) according to protocol provided by Asniwita (2013). 0.1 gram of leaf tissue was crushed in 1 ml of tris buffer saline (TBS) to produce sap. Then, 2 µl of sap per sample was dropped onto the nitrocellulose membrane. The positive and negative controls were used in DIBA detection. After the sap dried, the membrane was immersed in a blocking solution of non-fat milk in TBS containing Triton X–100 with a final concentration of 2% for 2 hours. The membrane was washed with dH_2O, then immersed in TBS containing 1 µl first antiserum (1:5,000 v/v) for 3 hours. The membrane was washed with TBST (TBS + tween 0.05%) and then the membrane was soaked in TBS buffer containing 1 µl second antibody (1:5,000 v/v) for 2 hours. The membrane washed again with TBST, then it was immersed in alkaline phosphatase buffer (pH 9.5) containing BCIP/NBT chromogenic substrates for 30 minutes until the color of positive samples on membrane change to purple. Further, the staining reaction was stopped by immersing the membrane in dH_2O. Detection result was used to determine the virus frequency (VF) according to the following formula:

$$VF = \frac{\text{Number of infected samples}}{\text{Total detected samples}} \times 100\%$$

Virus Detection using Polymerase Chain Reaction (PCR)

The samples with dark purple color by DIBA test from each location were selected for detection of nucleic acid by PCR. The total DNA was extracted from infected leaves using CTAB method (Doyle & Doyle, 1987) and the total DNA was diluted in 50 µl nuclease free water.

The amplification of total DNA was carried out by PCR using specific primer for ToLCNDV T-2F (5'-GGCATCTATTCTGAAACGACGTGG-3')/TS-2R (5'-CTTGGGTTTTCTGTTCATCGGCC-3') with amplicon size ± 600 bp and specific primer for SLCV S-2F (5'-CTATGGTATATTGGGGTATGG GGTC-3')/TS–2R with amplicon size ± 550 bp (Haerunisa, 2016). The amplification reaction for a total volume of 25 µl was 12.5 µl Go Taq green 2x (Thermo scientific), 1 µl of 10 µM reverse primer, 1 µl of 10 µM forward primer, 9.5 µl nuclease free water, and 1 µl of total DNA. DNA PCR product was separated on 1% agarose gel electrophoresis in 0.5X TBE buffer containing nucleic acid stain dye FlouroVue TM (Smobio, Taiwan). Electrophoresis was performed at 50 volts for 50 minutes and the DNA was visualized under UV transilluminator and documented by digital camera.

DNA Cloning and Sequencing

DNA cloning was conducted by TA-cloning of PCR product into pTZ57R/T vector plasmid (InsTAclone PCR Cloning Kit, Thermo Scientific). The cloning steps consisted of ligation, transformation, and confirmation of DNA plasmid. The ligation and transformation steps were performed according to protocol provided by Thermo Scientific.

The preparation of DH5α competent cells was carried out following the method by Sambrook & Russel (2001). The transformation was conducted by the chemical method (InsTAclone kit, Thermo Scientific). The *E. coli* DH5α bacterial pellet was dissolved in 300 µl T-solution (A-solution mixed with B–solution with a ratio of 150 µl/150 µl, v/v), then incubated on ice for 5 minutes. Bacterial culture was centrifuged at 3,000 rpm for 5 minutes and the pellet was collected and was diluted in 120 µl T solution, then it was incubated on ice for 5 minutes. The ligated DNA plasmid was taken 2.5 µl and added 50 µl of competent cells, then the mixture was homogenized and incubated on ice for 5 minutes. The DNA transformants were cultured in LB agar containing ampicillin (100 mg/L), 10 µl IPTG 100 mM, and 50 µl X–gal 2%. The bacterial culture was incubated at 37°C for 16 hours.

DNA plasmid carrying recombinant was confirmed by colony PCR of white colonies using universal primer pairs M13F (–20) and M13R (–26) pUC. The positive DNA plasmid containing target insert then cultured in LB–broth containing ampicillin and incubated at temperature 37°C on shaker at 150 rpm for overnight. Isolation DNA plasmid recombinant was carried out according protocol provided by Crosa *et al.* (1994). Bacterial cultures was centrifuged for 1 minute at 5,000 rpm and the supernatant was discarded. The bacterial pellet was added by 40 µl TE pH 8.0 and 600 µl lysis buffer (TE + 4% SDS pH 12.45), then incubated for 30 minutes at 37°C. Subsequently, the suspension was added by 30 µl Tris 2 M pH 7.0 and 240 µl NaCl 5 M, and incubated for 4 hours on ice. The suspension was centrifuged for 1 minute at 10,000 rpm. The supernatant was collected and transferred to a 1.5 ml micro tube, then added by equal volume of ethanol absolute and centrifuged for 15 minutes at 10,000 rpm. The supernatant was discarded, and the plasmid DNA pellet was washed by adding 500 µl 70% ethanol and centrifuged for 10 minutes at 10,000 rpm. Further, the 70% ethanol was discarded, and the plasmid DNA was dried for 30 minutes and then diluted in 50 µl TE buffer pH 8.0.

The plasmid DNA was send to First Base Malaysia for DNA sequencing. The matrix identity of nucleotide and amino acid was analyzed by BioEdit software, while the phylogenetic tree was constructed by MEGA V6.0 software using 1,000 times bootstrap.

RESULTS AND DISCUSSION

Symptoms and Disease Incidence in the Field

During field surveys in several cucumber cultivation areas in Java, we found varying virus-like symptoms on cucumbers such as yellow mosaic with leaf malformation, leaf chlorosis, upward curling of the leaf edges, yellowing with vein banding, leaf blister, and stunting (Figure 1). Previously, those types of symptoms were reported on Cucurbitaceae in India, Indonesia and Sri Lanka (Tiwari *et al*., 2010; Haerunisa *et al*., 2016; Sohrab *et al*., 2013).

Serological detection using specific antiserum of ToLCNDV and SLCV showed the average frequency of cucumbers positively reacted with ToLCNDV antiserum in East Java, Central Java, Yogyakarta, and West Java were 97.77%, 100%, 100%, and 92.77%, while SLCV were 93, 33%, 85.83%, 78.33%, and 89.99%, respectively (Table 1). It is indicated that the frequency of ToLCNDV and SLCV is very high detected on cucumbers in Java. ToLCNDV was previously reported to infect cucumber in Bali, Central Java, Yogyakarta, and West Java with disease incidence up to 100%, (Septariani *et al*., 2014; Haerunisa *et al*., 2016), while until present the existence of *Squash leaf curl China virus* (SLCCNV) found only in Bali with a disease incidence up to 80% (Wiratama *et al*., 2015).

ToLCNDV infection on cucumbers in East Java is the first report in this study. The highly frequency of ToLCNDV infection in the fields might due to highly whitefly population as vector with favorable environment temperature for the vector (25–40°C), and resistant cucumber varieties against ToLCNDV not available yet. The highly incidence of the disease is affected by the use of hybrid seeds throughout the growing season and the monoculture cultivation system (Maruthi *et al*., 2007). These facts in accordance with the conditions in the fields which are most farmers

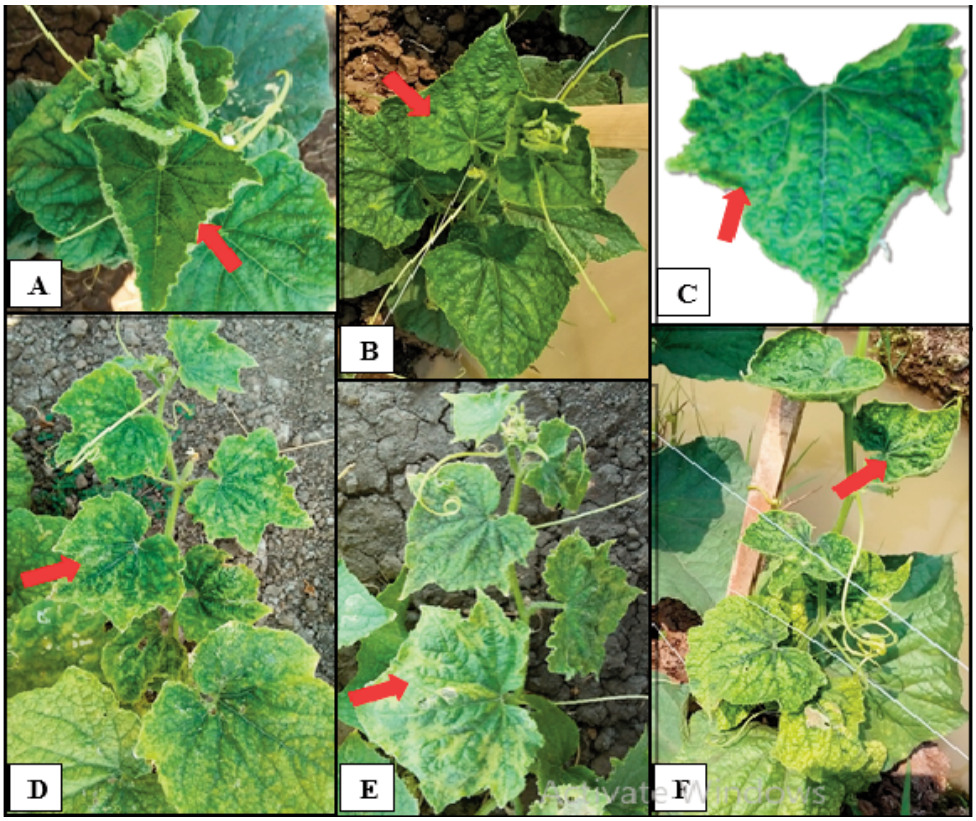

Figure 1. Symptoms of virus infection found on cucumbers in Java: upward curling of the leaf edge (A), mosaic (B), blister (C), yellowing leaves with green vein banding (D, E), chlorosis leaves and upward curling of the leaf edges with vein banding (F)

Table 1. The frequency of ToLCNDV and SLCV on cucumber in Java based on DIBA test

Location	Variety	Plant age (DAP)	Virus Frequency (%)	
			ToLCNDV	SLCV
Nganjuk	Harmoni	28–35	100	98.33
Kediri	Harmoni	33–37	93.33	81.66
Tulungagung	Harmoni	27–30	100	100
East Java	Average of virus frequency (%)		97.77	93.33
Brebes	Etana, Bangkok, Tami	32–44	100	93.33
Klaten	Kujangtirta	40–44	100	78.33
Central Java	Average of virus frequency (%)		100	85.83
Kulon Progo	Harmoni	30–37	100	78.33
Yogyakarta	Total of virus frequency (%)		100	78.33
Subang	Bandana, Sabana, Etana	30–34	100	98.33
Indramayu	Bandana, Sabana	28–40	80	71.66
Bogor	Bandana, Wulan	25–37	98.33	100
West Java	Average of virus frequency (%)		92.77	89.99

Remark: DAP = day after plantings

cultivate cucumber in monoculture system. In addition, the rapid spread of ToLCNDV in Java was facilitated by highly population of its vector *Bemisia tabaci* on cucumber, especially on underside leaves. Previously, Wiratama *et al.* (2015) reported that the high incidence of ToLCNDV and SLCCNV on cucumber in Bali might due to the high population of *B. tabaci*. Massive spread of ToLCNDV in cucumber in Java might be also caused by several factors, such as efficient ToLCNDV transmission by *B. tabaci* with persistent manner (one vector can transmit the virus to healthy plants), either virus or its vector have wide host range, and the presence of weeds surround cultivation area as reservoir plants as previously report by Tiwari *et al*. (2010).

Begomovirus *DNA Amplification by PCR*

Amplification of total DNA from samples positively reacted with ToLCNDV antiserum by PCR successfully amplified a DNA amplicon with size ±600 bp using a specific primer of ToLCNDV (Figures 2). However, samples positively reacted with SLCV antiserum did not amplified any DNA using SLCV specific primer, but positively amplified by ToLCNDV specific primer (data not shown). This indicates that SLCV antiserum cross-reacting against samples infected by ToLCNDV. The similarity of SLCV CP gene is closely to ToLCNDV (Haerunisa *et al*., 2016). The similar results occurred in *Soybean mosaic virus* (SMV) antiserum reacted positively against samples infected by *Bean common mosaic virus* (BCMV) on soybean (Rahim *et al*., 2015), and *Tomato spotted wilt virus* (TSWV) antiserum reacted positively with samples infected with *Chrysanthemum stem necrosis virus* (CSNV) (Boben *et al*., 2007). Cross-reaction serologically can occur between viruses species belongs to same genus.

The primer pair T-2F/TS-2R and S2F/TS-2R successfully amplified ToLCNDV and SLCV on cucumber samples in West Java and Bali, respectively (Haerunisa, 2016). However, in this study, SLCV did not amplify by specific primer of SLCV, indicating that SLCV might not present yet to infect cucumber in Java.

***Molecular Characterization of* Begomovirus**

The colony PCR of white colonies containing plasmid DNA of ToLCNDV confirmed that ToLCNDV successfully cloned. Ten ToLCNDV DNA clones from Nganjuk, Kediri, Tulungagung, Brebes, Klaten, Kulon Progo, Bogor, Indramayu, and Subang amplified the DNA with size ± 750 bp (Figure 3), which is consist of pTZ57R/T plasmid sequence ±150 bp and ToLCNDV DNA insert ± 600 bp.

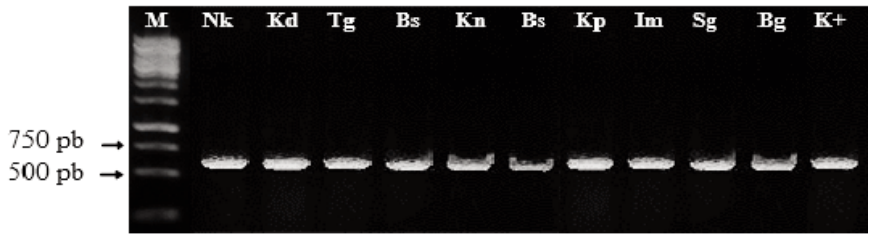

Figure 2. DNA visualization of ToLCNDV amplification using ToLCNDV-2F/ToLCNDV-2R primers; samples from Nganjuk (Nk), Kediri (Kd), Bogor (Bg), Kulon Progo (Kp), Brebes (Bs), Indramayu (Im), Tulungagung (Tg), Subang (Sg), and Klaten (Kn); M, 1 kb DNA marker (Thermo Scientific, USA)

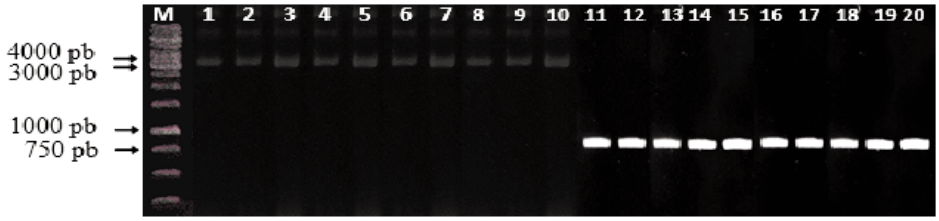

Figure 3. The DNA visualization of recombinant plasmid isolation from DH5α transformant bacteria (lanes 1–10); DNA bands of the CP ToLCNDV gene and PTZ57R/T vector which were separated after amplification using M13F (-20)/M13R (-26) pUC primers (lane 11–20); M, DNA marker 1 kb (Thermo Scientific, USA)

Homology of nucleotide and amino acid sequences among ToLCNDV Java isolates was ranging from 95.6–99.2% and 99.7–100%, respectively (Table 2). These results revealed that all ToLCNDV Java isolates are same species as previously stated by Brown *et al*., (2012) that the similarity of nucleotide sequences of *Begomovirus* isolate more than 89% belongs to the same virus species. The ToLCNDV Java isolates showed highest nucleotides (96.1–98.1%) and amino acids (99.7–100%) homology to Klaten isolates (AB613825) and lower homology to isolates from other countries (Table 2). Based on the sequences demarcation, *Begomovirus* is grouped in the same strain if the nucleotide sequences homology is more than 94% (Brown *et al*., 2012).

Phylogenetic analysis revealed that ToLCNDV Java isolates from Nganjuk, Kediri, Kulon Progo, Bogor, and Brebes are in the same cluster with ToLCNDV isolates from Klaten, Indonesia, and separated from ToLCNDV isolates that infected Cucurbitaceae from other Asian countries (Taiwan, Thailand, Japan, Bangladesh, Pakistan, India, and Iran) (Figure 4). DNA–A ToLCNDV from Klaten that infects cucumbers is classified as one strain of ToLCNDV, called ToLCNDV–Indonesia (Mizutani *et al*., 2011). ToLCNDV isolates from Indonesia that infect cucumbers are in one same cluster and those isolates separated distinctively to corresponding isolates from other countries. Based on the host plant, ToLCNDV Cucurbitaceae isolates are in a separate cluster from ToLCNDV Solanaceae isolates (Figure 4). These findings showed the existence of different ToLCNDV strains based on the area of origin and host plants. In addition, since the first report of ToLCNDV infection in Central Java by Mizutani *et al*., (2011), Septariani *et al*., (2014), Haerunisa *et al*., (2016), and also the present report clearly showed the rapid spreading of ToLCNDV in Java and Bali.

Table 2. Homology of nucleotides and amino acids of ToLCNDV Java isolates compared to corresponding isolates from other countries on GenBank

Origin of isolate	Homology (%)										Number of accessions
	Nganjuk		Kediri		Brebes		Kulon Progo		Bogor		
	nt	aa	nt	aa	nt	aa	nt	aa	nt	aa	
IDN - Ngjk	-	-	99.2	100	96.6	100	98.4	100	96.9	99.7	LC311791
IDN - Kdr	99.2	100	-	-	97.4	100	99.2	100	97.6	99.7	LC311792
IDN - Brbs	96.6	100	97.4	100	-	-	96.9	100	95.6	99.7	LC311793
IDN - KP	98.4	100	99.2	100	96.9	100	-	-	97.9	99.7	LC311790
IDN - Bgr	96.9	99.7	97.6	99.7	95.6	99.7	97.9	99.7	-	-	LC311789
IDN - Kla	97.6	100	98.4	100	96.1	100	97.6	100	98.1	99.7	AB613825
TAI	93.0	98.4	93.8	98.4	93.0	98.4	93.8	98.4	94.5	96.4	GU180095
THA1	93.5	98.4	94.3	98.4	94.0	98.4	93.8	98.4	94.0	96.4	AB330079
THA2	93.2	98.4	94.0	98.4	93.8	98.4	93.5	98.4	93.8	96.4	AB017342
JPN	93.2	98.2	94.0	98.2	93.8	98.2	93.5	98.2	93.8	96.4	AB020976
BD	94.5	96.4	95.5	96.4	95.1	96.4	95.3	96.4	95.8	96.4	KM383737
PAK1	94.3	96.4	95.1	96.4	95.1	96.4	94.6	96.4	94.5	94.6	EF620534
PAK2	94.0	94.6	94.8	94.6	94.8	94.6	94.3	94.6	94.3	94.6	LT556070
PAK3	93.8	94.6	94.5	94.6	94.8	94.6	94.0	94.6	94.5	96.4	DQ116885
PAK4	88.9	96.4	89.7	96.4	90.2	94.6	89.2	93.4	89.7	93.4	FN179278
IND1	93.5	96.4	94.3	96.4	94.3	94.3	93.8	96.4	94.3	94.8	U15016
IND2	91.2	96.4	92.0	96.4	92.5	94.3	91.5	94.6	91.4	94.6	AM286433
IND3	93.5	94.6	94.3	94.6	94.8	94.6	93.8	94.6	94.3	96.4	JX232220
IRN	90.9	96.4	91.7	96.4	91.7	93.4	91.2	94.6	91.7	93.4	KP641675
TLCKV*	54.3	65.3	54.3	65.3	54.3	65.3	53.7	65.3	54.1	61.5	KF446675

Remarks: TLCKV* = *Tomato yellow leaf curl Kanchanaburi virus* Indonesian isolate infected chili as an out group; nt (nucleotide) and aa (amino acid); IDN = Indonesia, TAI = Taiwan; THA = Thailand, JPN = Japan, BD = Bangladesh, PAK = Pakistan, IND = India, IRN = Iran.

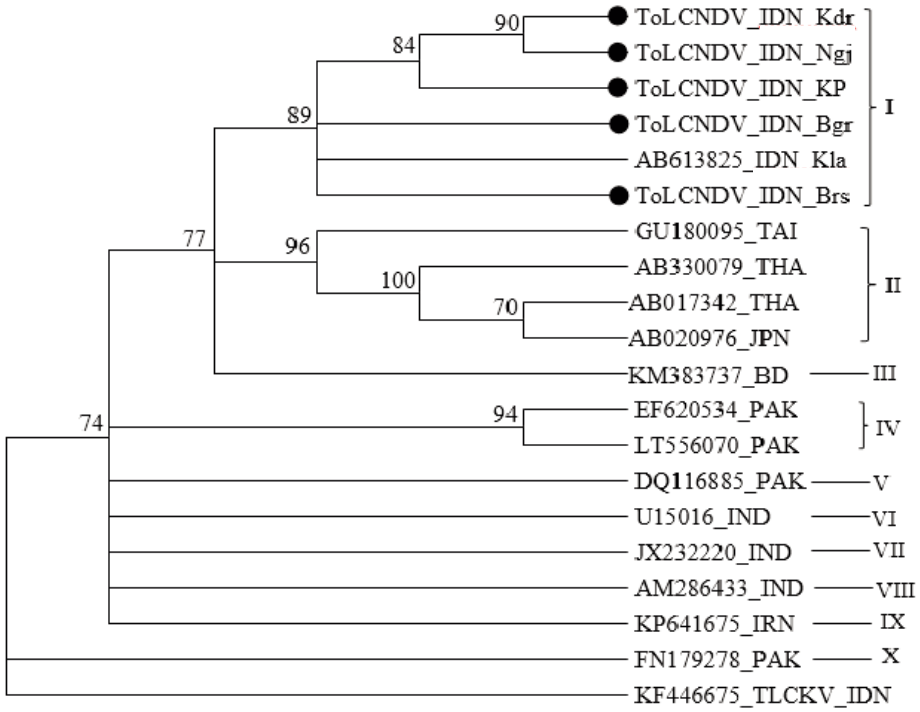

Figure 4. Phylogenetic tree of CP ToLCNDV gene from Javanese isolates compared to 14 ToLCNDV isolates in Genbank; *Tomato yellow leaf curl Kanchanaburi virus* (TLCKV) from Indonesia is used as out group; isolates marked with black dots are Java isolates; IDN–Indonesia, PAK–Pakistan, TAI–Taiwan, THA–Thailand, JPN–Japan, BD–Bangladesh, IND–India, IRN–Iran, Ngj–Nganjuk, Kdr–Kediri, KP–Kulon Progo, Bgr–Bogor, Brs–Brebes, Kla–Klaten

CONCLUSION

ToLCNDV was successfully detected with highly frequency from cucumber cultivations in Java. The ToLCNDV distributes evenly, while SLCV is not detect yet in Java. Phylogenetic analysis of nucleotide sequences revealed that the ToLCNDV Java isolates from Nganjuk, Kediri, Kulon Progo, Bogor, and Brebes are in the same cluster with ToLCNDV isolates from Klaten, Indonesia and separated distinctively to corresponding isolates from other countries.

ACKNOWLEDGEMENT

The research was funded by the Indonesia Ministry of Research and Technology and Higher Education for TAD and team through PMDSU scheme and postgraduate scholarship for the first author with contract no. 330/SP2H/LT/DRPM/IX/2016 and 136/SP2H/LT/DRPM/IV/2017.

LITERATURE CITED

Ali-Shtayeh, M.S., R.M. Jamous, E.Y. Hussein, O.B. Mallah, & S.Y. Abu–Zeitoun. 2014. *Squash leaf curl virus* (SLCV): A Serious Disease Threatening Cucurbits Production in Palestine. *Virus Genes* 48: 320–328.

Asniwita. 2013. *Karakterisasi* Chili veinal mottle virus *Strain Lemah dan Potensinya sebagai Agens Proteksi Silang*. Disertasi, Institut Pertanian Bogor. Bogor. 24 p.

Bandaranayake, W.M.E.K., W.A.R.T. Wickramarachchi, H.A.M. Wickramasinghe, R.G.A.S. Rajapakshe, & D.M.K.K. Dissanayake. 2014. Molecular Detection and Characterization of *Begomoviruses* Associated with Cucurbitaceae Vegetables in Sri Lanka. *Journal of the National Sciences Foundation Sri Lanka* 42: 265–271.

Boben, J., N. Mehle, M. Pirc, P.I. Mavric, & M. Ravnikar. 2007. New Molecular Diagnostic Methods for Detection of *Chrysanthemum stem necrosis virus* (CSNV). *Acta Biologica Slovenica* 50: 41–51.

BPS [Badan Pusat Statistik]. 2017. *Produksi Tanaman Sayuran di Indonesia Tahun 2012–2016*. Badan Pusat Statistik Republik Indonesia, Jakarta. 135 p.

Brown, J.K., A.M. Idris, C. Alteri, & D.C. Stenger. 2002. Emergence of A New Cucurbit-Infecting *Begomovirus* Species Capable of Forming Viable Reassortants with Related Viruses in the *Squash leaf curl Virus* Cluster. *Phytopathology* 92: 734–742.

Brown, J.K., C.M. Fauquet, R.W. Briddon, M. Zerbini, E. Moriones, & J.N. Castillo. 2012. Family Geminiviridae, p. 351–373. *In* M.Q.A. Kings, M.J. Adams, E.B. Carstens, E.J. Lefkowitz, (eds.), *Virus Taxonomy: Ninth Report of the International Committee on Taxonomy of Viruses.* Elsevier, Inc. London.

Crosa, J.H., M.E. Tolmasky, L.A. Actis, & S. Falkow. 1994. Methods for General and Molecular Bacteriology. *American Society for Microbiology* 176: 365–386.

Doyle, J.J. & J.L. Doyle. 1987. A Rapid DNA Isolation Procedure for Small Quantities of Fresh Leaf Tissue. *Phytochemical Bulletin* 19: 11–15.

Haerunisa, R. 2016. *Identifikasi dan Desain Primer Diferensial Dua* Begomovirus *pada Mentimun di Jawa Barat dan Bali*. Tesis. Institut Pertanian Bogor, Bogor. 16 p.

Haerunisa, R., G. Suastika, & T.A. Damayanti. 2016. Identifikasi *Begomovirus* yang Berasosiasi dengan Penyakit Kuning pada Mentimun di Jawa Barat dan Bali. *Jurnal Hortikultura Indonesia* 7: 9–20.

Jones, D.R. 2003. Plant Viruses Transmitted by Whiteflies. *European Journal of Plant Pathology*. 109: 195–219.

Khameneh, S.Y., S. Aboutorabi, M. Shoori, A. Aghazadeh, P. Jahanshahi, A. Golnaraghi, & M. Maleki. 2016. Natural Occurrence of *Tomato leaf curl New Delhi virus* in Iranian Cucurbit Crops. *The Plant Pathology Journal* 32: 201–208.

Maruthi, M.N., A.R. Rekha, & V. Muniyappa. 2007. Pumpkin Yellow Vein Mosaic Disease is Caused by Two Distinct *Begomoviruses*: Complete Viral Sequences and Comparative Transmission by an Indigenous *Bemisia tabaci* and the Introduced B-Biotype. *Bulletin OEPP/EPPO Bulletin* 37: 412–419.

Mizutani, T., B.S. Daryono, M. Ikegami, & K.T. Natsuaki. 2011. First Report of *Tomato leaf curl New Delhi virus* Infecting Cucumber in Central Java, Indonesia. *Disease Notes* 95: 1485.

Phaneedra, C., K.R.S.S. Rao, R.K. Jain, & B. Mandal. 2012. *Tomato leaf curl New Delhi virus* is Associated with Pumpkin Leaf Curl: A New Disease in Northern India. *Indian Journal of Virology* 23: 42–45.

Pratap, D., A.R. Kashikar, & S.K. Mukherjee. 2011. Molecular Characterization and Infectivity of A *Tomato leaf curl New Delhi virus* Variant Associated with Newly Emerging Yellow Mosaic Disease of Eggplant in India.*Virology Journal* 8: 305–312.

Rahim, Y.F., T.A. Damayanti, & M. Ghulamahdi. 2015. Deteksi Virus yang Menginfeksi Kedelai di Jawa. *Jurnal Fitopatologi Indonesia* 11: 59–67.

Sambrook, J. & D.W. Russel. 2001. *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, New York. 1881 p.

Septariani, N.D., S.H. Hidayat, & E. Nurhayati. 2014. Identifikasi Penyebab Penyakit Daun Keriting Kuning pada Tanaman Mentimun. *Jurnal Hama dan Penyakit Tumbuhan Tropika* 14: 80–86.

Sohrab, S.S., B. Mandal, A. Ali, & A. Varma. 2013. Chlorotic Curly Stunt: A Severe *Begomovirus* Disease of Bottle Gourd in Northern India. *Indian Journal of Virology* 21: 56–63.

Tiwari, A.K., P.K. Sharma, M.S. Khan, S.K. Raj, & G.P. Rao. 2010. Molecular Detection and Identification of *Tomato leaf curl New Delhi virus* Isolate Causing Yellow Mosaic Disease in Bitter Gourd (*Momordica charantia*), A Medicinally Important Plant in India. *Medicinal Plants* 2: 117–123.

Venkataravanappa, V., P. Swarnalatha, R.C.N. Lakshminarayana, B. Mahesh, & A.B. Rai. 2012. Molecular Evidence for Association of *Tobacco curly shoot virus* and a Betasatellite with Curly Shoot Disease of Common Bean (*Phaseolus vulgaris* L.) from India. *Journal of Plant Pathology and Microbiology* 3: 148.

Wilisiani, F., S. Somowiyarjo, & S. Hartono. 2014. Identifikasi Molekuler Virus Penyebab Penyakit Daun Keriting Isolat Bantul pada Melon. *Jurnal Perlindungan Tanaman Indonesia* 18: 47–54.

Wiratama, I.D.M.P., G.N.A.S. Wirya, N.N.P. Adnyani, I.D.N Nyana, & G. Suastika. 2015. Temuan Penyakit Baru: Laporan Pertama Infeksi *Begomovirus* pada Tanaman Mentimun di Bali. *Jurnal Fitopatologi Indonesia* 11: 175–178.