

## Detection and identification of *Begomovirus* infecting Cucurbitaceae and Solanaceae in Yogyakarta, Indonesia

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**Abstract.** *Subiastuti AS, Hartono S, Daryono BS. 2019. Detection and Identification of Begomovirus infecting Cucurbitaceae and Solanaceae in Yogyakarta, Indonesia. Biodiversitas 20: 738-744.* *Begomovirus* genome has high plasticity that led to evolve rapidly. *Begomovirus* is one of a remarkably successful group of emerging viruses as the results from combination of many factors. Planting systems in Indonesia which often overlapping two or more plant species in one land has high possibility for occurring mixed infection. It is also suggested has high contribution to increase *Begomovirus* diversity. The aim of this research is to do preliminary identification of *Begomovirus* infected-Solanaceae and Cucurbitaceae in Yogyakarta based core coat protein (CP) gene sequence. A total of 50 melon, 50 chili, 30 eggplants, and 30 watermelon samples which showed *Begomovirus* symptoms were observed from several fields in Yogyakarta and Purworejo, Indonesia during 2016. Almost 90% of infected samples for each plant were tested by PCR and showed positive for *Begomovirus*. Based on coat protein (CP) gene nucleotide sequence identity, *Begomovirus* infected Solanaceae in Indonesia has close relationship with *Pepper yellow leaf curl Indonesia virus* (PepYLCIV) and *Tomato yellow leaf curl Kanchanaburi virus* (TYLCVKaV), while in Cucurbitaceae has close relationship with *Squash leaf curl China virus* (SLCCV) *Tomato leaf curl New Delhi virus* (ToLCNDV). All collected isolates showed highest sequence identity with isolates from South-East Asia and China. However, further analysis that including full genome characterization is still needed to explain *Begomovirus* evolution in Indonesia.

**Keywords:** *Begomovirus*, coat protein, Cucurbitaceae, genetic variation, Solanaceae

### INTRODUCTION

*Begomovirus* is DNA virus that characterized with monopartite or bipartite circular single-stranded DNA genomes encapsidated in twinned isohedral particles. The size of genome is approximately 2600 nt for each of them (Fauquet and Stanley 2003). Infected plants exhibit several symptoms including stunting and leaf distortion and some combination of golden-light green-yellow mosaic/mottle, leaf curling, crumpling, veinal or interveinal yellowing, and yellow spots in leaves (Inoue-Nagata et al. 2016).

*Begomovirus* is one of a remarkably successful group of emerging viruses (Bridson et al. 2010; Rojas et al. 2008). This is as the results from combination many factors. *Begomovirus* is transmitted by *Bemisia tabaci*, one of the most invasive polyphagous vector that can infect many crops. High population of *B.tabaci* is directly correlated with the emergence or introduction of new *Begomovirus* species in new area. Moreover, *Begomovirus* genome has high plasticity that led to evolve rapidly. *Begomovirus* genome can encounter mutation, pseudorecombination, recombination, and acquisition of new DNA components and satellites that leading to high infection rates, expanded host range, evolved more virulence strains, and more adapted in environment changed (Seal et al. 2006; Saunders et al. 2002).

*Begomoviruses* cause various diseases of dicotyledonous crops in temperate and tropical zones of the

world. Severe yield losses have been reported due to *Begomovirus* in Indonesia especially in Cucurbitaceae and Solanaceae. Three Cucurbit-infecting begomoviruses have been reported in Java Island; *Squash leaf curl Philippines virus* (Julijantono et al. 2010), *Tomato yellow leaf curl New Delhi virus* (Mizutani et al. 2011), *Pepper yellow leaf curl Indonesia virus* (Wilisiani et al. 2014). While in Solanaceae, there are many reports about *Begomovirus*-infection in Indonesia, especially in Tomato and Pepper, such as *Tomato leaf curl Java virus* (Kon et al. 2006), *Tomato leaf curl Philippine virus*, *Pepper yellow leaf curl Indonesia virus* (Sakamto et al. 2005), *Ageratum yellow vein virus* (Tsai et al. 2009), and *Tomato yellow leaf curl Kanchanaburi virus* (Kenyon et al. 2014). However, the data about *Begomovirus* infection in South-East Asia including Indonesia, is still very limited.

Based on complete nucleotide sequences of the genome, *Begomovirus* is classified into two groups, bipartite and monopartite. Bipartite *Begomovirus* consists of two circular ssDNA genome, DNA A and DNA B while monopartite *Begomovirus* lack of DNA B. DNA A possesses six open reading frame (ORFs): AV1, AV2, AC1, AC2, AC3, and AC4 protein. AV2 is only found in old world begomoviruses (Snehi et al. 2017). AV1 is coat protein (CP) that play role at ssDNA encapsidation, virus particle formation, viral movement, and vector transmission (Snehi et al. 2017, Seal et al., 2006, Poonam et al. 2017). DNA B has two ORFs, BV1 and BC1. BV1

encodes nuclear shuttle protein and BC1 encodes movement protein. The 50 ~ 200 nucleotides (nt) of the CP gene are highly variable and useful as an informative region for predicting taxonomic relationships within the genus *Begomovirus*. When complete genome or component of bipartite virus sequences are unavailable, full-length CP gene sequences can be used for rapid detection followed by prediction of *Begomovirus* species identification (Brown et al. 2001; Mayo and Pringler 1997; Palmieri et al. 2003). CP was used to analyze genetic diversity of begomoviruses infecting native and cultivated plants from Mexico (Hernandez-zepeda et al. 2007), soybean and bean in Argentina (Rodriguez-Pardina et al. 2006), and cotton in India (Sharma et al. 2005). However, based on the International Committee of Virus Taxonomy (ICTV) guideline, the complete DNA-A sequence or monopartite genome sequence are needed for definite identification of *Begomovirus* species (Hernandez-Zepeda et al. 2007).

Planting systems in Indonesia which often overlapping two or more plant species in one land has high possibility for occurring mixed infection. It is also suggested has high contribution to increase *Begomovirus* diversity. Overlapping planting system also provides continual host plants for whitefly population which always close correlated with virus emergence (Brown et al. 2001). The aim of this research is to describe the provisional genetic variation of *Begomovirus* infecting members of Solanaceae and Cucurbitaceae in Yogyakarta, Indonesia based on CP gene sequence. The result will be useful in developing strategy to control *Begomovirus* infection in Indonesia.

## MATERIALS AND METHODS

### Sample collection and DNA isolation

Observation was conducted in agriculture area where several types of plants planted together. Infected plants showed *Begomovirus* symptoms including, stunting, leaf curling, foliar mosaic, were collected from Sleman, Bantul, and Kulonprogo (Yogyakarta, Indonesia), as well as Purworejo (Central Java, Indonesia) during 2017-2018 (Table 1). All locations located near each other (Figure 1). Total nucleic acids were extracted from leaf samples using Plant DNA Extraction kit described by Daryono and Natsuaki (2002). Before used as PCR sample, DNA was checked quantitatively using spectrophotometry with absorbance 260/280 nm.

**Table 1.** Plant species and geographic area of field-collected samples selected for detection and identification of *Begomovirus*

Sample code	Plant species	Field location	
		Sub-district, district	Province
Bnt 3	Melon	Bantul, Bantul.	Yogyakarta
Bnt 9	Melon	Palpabang, Bantul	Yogyakarta
Bnt 11	Pepper	Bambanglipuro, Bantul	Yogyakarta
KP3	Chili	Purwosari, Kulonprogo	Yogyakarta
KP4	Chili	Purwosari, Kulonprogo	Yogyakarta
KP5	Chili	Wates, Kulonprogo	Yogyakarta
PWJ 2	Chili	Ngombol, Purworejo	C. Java
PWJ 8	Watermelon	Ngombol, Purworejo	C. Java
PWJ 15	Watermelon	Ngombol, Purworejo	C. Java
SLM 1	Eggplant	Berbah, Sleman	Yogyakarta
SLM 2	Eggplant	Berbah, Sleman	Yogyakarta
SLM 6	Eggplant	Berbah, Sleman	Yogyakarta
SLM 8	Melon	Kalitirto, Sleman	Yogyakarta
SLM 9	Melon	Jamusan, Sleman	Yogyakarta
SLM10	Melon	Kalitirto, Sleman	Yogyakarta



**Figure 1.** Study site in Yogyakarta and Central Java, Indonesia. 1. Ngombol, Purworejo; 2. Wonosari, Kulonprogo; 3. Wates, Kulonprogo; 4. Palpabang, Bantul; 5. Bambanglipuro, Bantul; 6. Bantul, Bantul; 7. Berbah, Sleman; 8. Kalitirto, Sleman; 9. Jamusan, Sleman

### Amplification of Coat Protein Gene

Initially, PCR using Krusty Homer universal primers (Krusty (Forward): 5'CCNMRDGGHTGTGARGGNCC'3; Homer (Reverse): 5'SVDGCRTGVGTRCANGCCAT'3) were used to amplify a part of coat protein gene of begomoviruses. This primer produces ~550 bp DNA band (Revell et al. 2003). PCR mix was used in 25 µL containing 12.5 µL PCR kit Ready Mix, 1.25 µL for each primer (10 pmol), 2 µL DNA samples (200 ng), and 8 µL distilled water. PCR reaction began with initial denaturation at 95° C for 5 minutes, continue with 35 cycles of 95° C for 30 seconds, 55° C for 30 seconds, and 72° for 45 seconds then followed by final extension at 72° C for 5 minutes. The PCR results were analyzed using 1.2% agarose gel electrophoresis staining with Florosafe DNA stain in Tris Borate EDTA (TBE) buffer.

### DNA Sequence Analysis

Specific bands were isolated from agarose gel using gel purification kit then sequenced using ABI3730xl DNA Sequencer. DNA sequences were assembled using Genetyx Ver\_10, then sequence similarity searches were carried out by comparing sequences to other coat protein gene sequences of *Begomovirus* in GenBank using BLAST. Phylogenetic trees were constructed at maximum likelihood algorithm with 2000 bootstraps using MEGA 7.0, then edited manually (Islam et al. 2018).

## RESULTS AND DISCUSSION

A total of 50 melon, 50 chili, 30 eggplants, and 30 watermelon samples which showed *Begomovirus* symptoms were observed from several fields in Yogyakarta and Purworejo, Indonesia during 2016. Purworejo is a district in Central Java Province which is located near

Kulonprogo, Yogyakarta. This area is central to watermelon field in Yogyakarta and Central Java. Almost 90% of infected samples for each plant were tested by PCR and showed positive for *Begomovirus* infection.

Based on sampling location, three samples from each location were sequenced (Table 2) except for Sleman. Six samples consist of 3 melon samples and 3 eggplant samples from Sleman were analyzed because this area is university research field where several cultivars from several cities in Indonesia were planted whole year for research purpose.

BLASTn analysis was conducted using CP gene partial sequence. The result showed different *Begomovirus* for each area and each type of plants. All samples analyzed showed more than 94% sequence identity with > 470 bp sequence of coat protein gene of *Begomovirus* in GenBank. Two melon samples from Bantul displayed more than 97% sequence identity with *Squash leaf curl China virus* (SLCCV) while chili sample has 98% sequence identity with *Pepper yellow leaf curl Indonesia virus* (PepYLCIV). Samples from Kulonprogo showed that chili field on this area was infected with *Tomato yellow leaf curl Kanchanaburi virus* (TYLCKaV) and *Pepper yellow leaf curl Indonesia virus* (PepYLCIV). Kulonprogo is directly adjacent to Purworejo. Chili samples from Purworejo showed 94% sequence identity with *Pepper yellow leaf curl Indonesia virus* (PepYLCIV) same with sample from Kulonprogo. In the other hand, watermelon samples from Purworejo displayed more than 96% identity with *Squash leaf curl China virus* (SLCCV). All of eggplant samples displayed 99% sequence identity with *Tomato yellow leaf curl Kanchanaburi virus* (TYLCKaV), while melon samples showed infection from two different *Begomovirus*. Two melon samples showed more than 97% sequence identity with *Tomato leaf curl New Delhi virus* (ToLCNDV) and one sample showed 95% sequence identity with *Squash leaf curl China virus* (SLCCV).

**Table 2.** Sequence identity of partial coat protein gene of each sample and the species with higher identity

Sample	% Identity	<i>Begomovirus</i>	GenBank Accession
Bnt 3	97	Squash leaf curl China virus (SLCCV)	EF197940
Bnt 9	97	Squash leaf curl China virus (SLCCV)	EF197940
Bnt 11	98	Pepper yellow leaf curl Indonesia virus (PYLCInV)	KX900941
KP3	99	Tomato yellow leaf curl Kanchanaburi virus (TYLCKaV)	KF446675
KP4	99	Tomato yellow leaf curl Kanchanaburi virus (TYLCKaV)	KF446675
KP5	96	Pepper yellow leaf curl Indonesia virus (PYLCInV)	AB267838
PWJ 2	94	Pepper yellow leaf curl Indonesia virus (PYLCInV)	AB267838
PWJ 8	98	Squash leaf curl China virus (SLCCV)	EF197940
PWJ 15	97	Squash leaf curl China virus (SLCCV)	EF197940
SLM 1	99	Tomato yellow leaf curl Kanchanaburi virus (TYLCKaV)	KF446675
SLM 2	99	Tomato yellow leaf curl Kanchanaburi virus (TYLCKaV)	KF446675
SLM 6	99	Tomato yellow leaf curl Kanchanaburi virus (TYLCKaV)	KF446675
SLM 8	98	Tomato leaf curl New Delhi virus (ToLCNDV)	AB613825
SLM 9	95	Squash leaf curl China virus (SLCCV)	EF197940
SLM 10	97	Tomato leaf curl New Delhi virus (ToLCNDV)	AB613825

Phylogenetic analysis was conducted by comparing sample sequences with *Begomovirus* coat protein gene sequences from *GenBank* (Figure 2). Comparative sequences were *Begomovirus* infecting plants in Southeast Asia, South Asia, and China. Phylogenetic tree displayed into four clades. Clade A and B were group of *Begomovirus* infecting Solanaceae, while clade C and D were group of *Begomovirus* infecting Cucurbitaceae. Clade A is group of TYLCKaV. Three samples from eggplant and two samples from chili were clustered together with TYLCKaV Indonesia's isolates. PePYLCIV was clustered in clade B. PWJ2 and KP5 clustered with PepYLCIV ageratum isolate while BNT 11 clustered with PepYLCIV chili isolate. On clade C, there were only 2 samples that clustered together with ToLCNDV. They showed highest identity of partial CP gene with Indonesia isolate and separated with ToLCNDV from Thailand, Bangladesh, and India. While in clade D samples BNT 3, BNT9, SLM9, PWJ 2, and PWJ 15 were clustered together with SLCCV isolate Malaysia but in different subclade with isolate from China, Taiwan, and Philippines.

*Begomovirus* infection in Indonesia was first reported in West Java in 1999 and spread into Central Java in 2003. First infection was PePYLCIV in Pepper (Rusli et al. 1999; Sulandari 2006). While in 1998, tomato-infecting *Begomovirus* was found in Lembang and named as *Tomato yellow leaf curl Indonesia virus* (TYLCIDV) (Tsai et al. 2006). It was first report about *Begomovirus* infecting member of Solanaceae in Indonesia. Then in 2006, *Tomato leaf curl Java virus* (ToLCJaV) was reported infecting tomato in Bogor (Kon et al. 2006). However, there was no further report about TYLCIDV and ToLCJaV infection in Indonesia crops after that. *Begomovirus* infecting Solanaceae developed into *Tomato yellow leaf curl Kanchanaburi virus* (TYLCKaV). TYLCKaV isolate from Indonesia was first found in eggplant and very similar to that initially identified in Thailand (Kenyon et al. 2014). Our results indicated that PePYLCIV and TYLCKaV were main cause of severe infection among the members of Solanaceae at Yogyakarta.

*Begomovirus* was also found infecting Cucurbitaceae in Indonesia. Cucurbit-infecting *Begomovirus* was first reported caused by *Squash leaf curl Philippines virus* (SLCPV). That infection occurred in melon at Central Java and Yogyakarta (Julijantono et al. 2008). Different *Begomovirus* species infecting cucurbit, ToLCNDV, was found during 2011 (Mizutani et al. 2011). Then in 2014, PepYLCIV was also known infecting melon in Bantul, Yogyakarta (Wilisiani et al. 2014). Scientific data about *Begomovirus* infected Cucurbitaceae is still limited. From three species which have been reported, only full genome of ToLCNDV that have been characterized. Although our results showed that there were no new species of *Begomovirus* that infecting members of Cucurbitaceae in Yogyakarta, we suppose that there are still many begomoviruses infecting cucurbits that have not been identified. It is because of high incidence of *Begomovirus* infection at almost all province in Indonesia.

*Bemisia tabaci*, as vector of *Begomovirus*, has important role in the spread of infection. *B. tabaci* biotype

B is known as highly polyphagous and rapidly develop pesticide resistance. This biotype is invasive. It can displace indigenous whitefly and often results in severe virus outbreaks when introducing to new area (Pan et al. 2012; Sealet et al. 2006). Coat protein (CP) located on the surface of *Begomovirus* virion is key of *Begomovirus* translocation in *B. tabaci*. The CP will contact with insect tissue to allow virion bind to insect putative receptors and determine which biotype can carry them (Czoznek et al. 2017).

While in *Begomovirus*, CP has role in encapsidated of viral DNA. Mutants with lack of CP protein accumulate low levels of ssDNA as result from lack of ssDNA protection from enzymatic degradation (Sunter and Bisaro 1991). CP also the most conserved region of *Begomovirus* genome. Comparison of CP sequence can be used to identify how *Begomovirus* genetic relationship. Because of CP important role viral DNA encapsidation, CP also has role in viral movement within plant as well as in vector transmission (Snehi et al. 2017). Mutation of CP is closely related to *Begomovirus* variation. High sequence identity of CP can led conclusion that *Begomovirus* from same area has closest relationship.

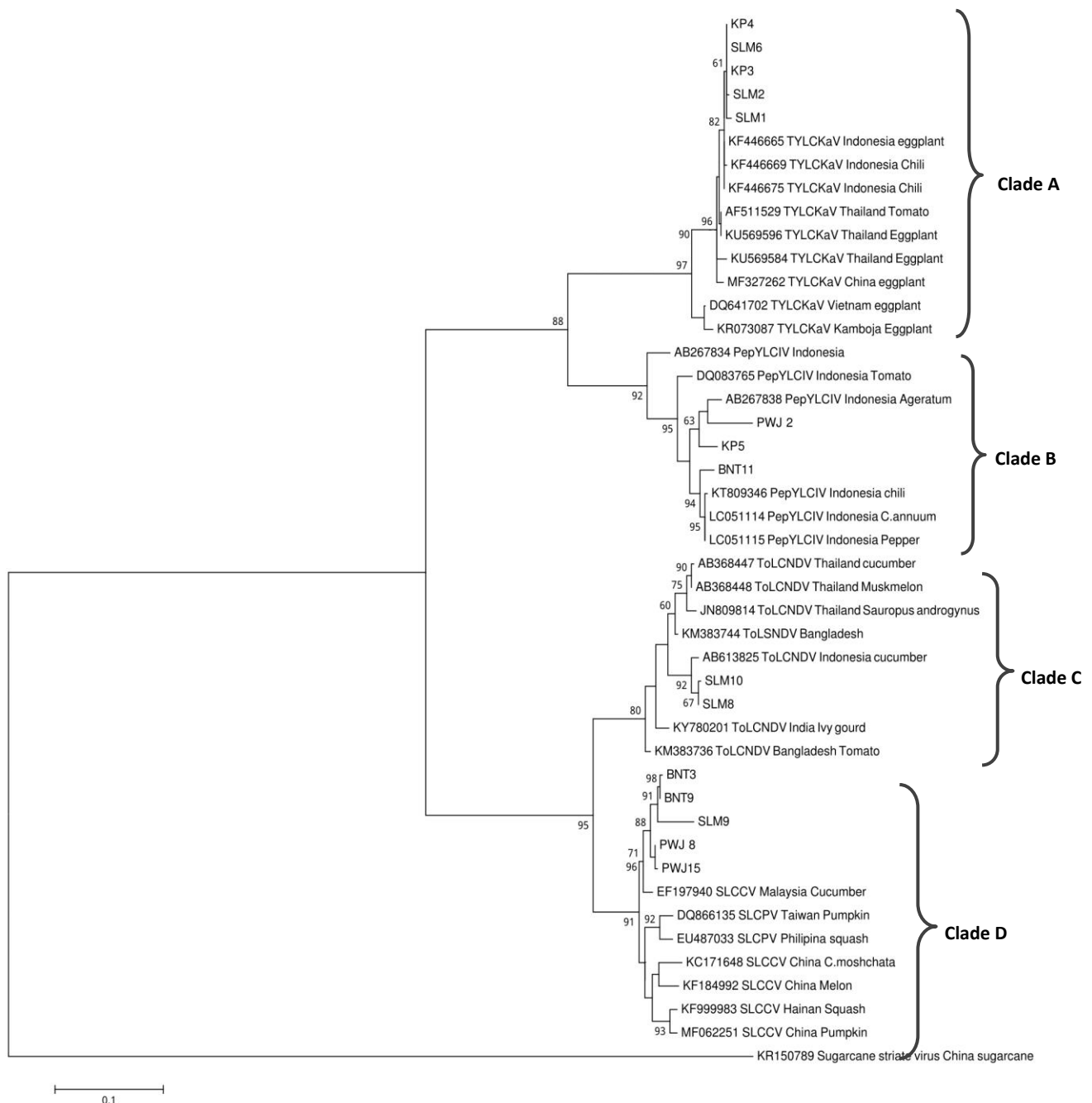
Krusty Homer primer was amplified region of 460-1000 nucleotides of CP gene results ~550 bp. This nucleotide covers conserved and variable regions of CP gene (Revill et al. 2003). Based on CP partial sequences, our results showed that begomoviruses clustered into 2 main groups based on host plants, Solanaceae and Cucurbitaceae. Phylogenetic tree showed relationship between sample isolate with closest species based on CP sequence identity (Figure 2). Begomoviruses infecting members of Solanaceae in Indonesia showed high similarity (of CP sequence) with isolate from Indonesia than China and other countries in South-East Asia. TYLCKaV infecting members of Solanaceae in Yogyakarta and Central Java showed high similarity with isolate from Indonesia, Thailand, and China but separated from Vietnam and Kamboja isolate. While PepYLCIV showed different pattern. PepYLCIV infecting members of Solanaceae in Yogyakarta and Purworejo separated into three groups based on its host, ageratum and chili. In Cucurbitaceae groups, two samples clustered with ToLCNDV Indonesia isolate and separated from ToLCNDV from Thailand, Bangladesh, and India. However, SLCV showed that Indonesia isolate has high similarity with Malaysia isolate but not similar enough with China, Philippine, and Taiwan isolate. SLCV evolutionary pattern may be caused by host introduction by human. However, this results only describe genetic similarity of CP gene sequence of *Begomovirus*. CP sequences can be used for rapid detection of *Begomovirus* infection and preliminary identification of *Begomovirus* in new area. Full sequences of DNA-A are needed for establishing new species and definite classification (Hernandez-zepeda et al. 2007). A partial or complete sequences of CP gene has been used to establish preliminary identification of begomoviruses associated with native flora and cultivated plants in Mexico (Hernandez-Zepeda et al. 2007), *Begomovirus* infecting mungbean in Bangladesh (Islam et al. 2002), and for study of genetic diversity of *Begomovirus*

in Pakistan (Islam et al. 2018).

Variation of CP implies to *Begomovirus* evolution. As key of vector transmission and viral movement in plant, CP proteins have evolved differently in different geographic areas and different host. However, region-specific selection pressure has a greater influence than plant host range (Harrison and Robinson 1999). Before transmitted into new area, *Begomovirus* was selected by its vector then by host range. During vector transmission, only a limited amount of virus can be acquired by vector so that not all virus population present in the host plant will be transmitted. The prevailing *B. tabaci* biotypes present in a region will affect the begomoviruses present. Most biotypes can transmit a range of begomoviruses, but they do so with very different

efficiencies depending on both virus species and vector biotype (Bedford et al. 1994; McGrath and Harrison 1995; Maruthi et al. 2002). This phenomenon implies to strong co-adaptation between vector and virus.

When virus has been transmitted by vector to new host, second selection pressure will occur. Host plant specificity are different among species depend on virus genome plasticity and plant resistance mechanisms (Moriones and Navas-Castillo 2000, Seal et al. 2006). It also displayed in Figure 2 that some species of *Begomovirus* only found infected members of Solanaceae and others only infected members of Cucurbitaceae. It can be one of reason why phylogenetic separated into two main clades based on host plants.



**Figure 2.** Genetic variation of CP sequences between *Begomovirus* isolated from several agriculture fields in Yogyakarta with identified begomoviruses isolate from *GenBank*

There are many sources of genetic variation in begomoviruses. The occurrence of mutation, pseudo-recombination, and recombination also play important role besides selection by vector and plant host. In DNA virus, mutation should have lower effect than recombination because of DNA-polymerase activity. However, some geminiviruses allow non-deleterious mutations to be maintained because they do not utilize normal host mechanism for mismatch repair (Roosnick 1997). Pseudo-recombinations have been reported to occur on several begomoviruses (Garrido-Ramirez et al. 2000; Pita et al. 2001; Idris et al. 2003; Ramos et al. 2003). *Begomovirus* has ability to make associations between DNA-A component and DNA-B from another virus. Some monopartite *Begomovirus* can acquire DNA B permanently and changing to bipartite virus (Saunders et al. 2002). Unlike with pseudorecombination, the process by which from one nucleotide strand become incorporated into different individual strand is known as recombination. Recombination often occurs on nucleotide 2600 - 140 that contain intergenic region (IR). Some evidence showed that overlapping regions including AV2/AV1, AV1/AC3, and AC3/AC2 junctions also appear as recombination hotspot in *Begomovirus* genome (Kirthi et al. 2002; Seal et al. 2006). Recombinant viruses are generally more adapted to plant host defense as a result of changes in IR or overlapping genes (Eagle et al. 1994; Chellapan et al. 2004).

Overlapping planting system in Indonesia facilitated greater prevalence for mixed infections occurs. Mixed infections are precondition for recombination. Even though continuous overlapping cropping system is mostly done by Indonesian farmers, data about *Begomovirus* infection is still very limited. This indicates that recombination events in *Begomovirus* population still not recorded well. Figure 2 indicates that SLM 9 has different branches than other SLCV melon isolates found. SLM 9 was isolated from areas previously planted with chili and located around cucumber field that are almost harvested, so that there is a possibility of a mixture of infection or recombination. Further analysis of full genome characterization is needed for this isolate.

Preliminary identification of *Begomovirus* in some area can provisionally describe the potential evolution of *Begomovirus* and the effect of cropping patterns on the diversity of *Begomovirus* population. The main factor that causes *Begomovirus* diversity develop rapidly is a condition that allows the genome rearrangements. Global seed trade also has role in accelerating *Begomovirus* evolution by facilitating species to reach new area as well as introducing vectors to different host preferences. Control of *Begomovirus* needs integrated solutions to minimize the emergence of viruses and vector populations. Furthermore, crop management must reduce selection factors that increase the more virulent virus or more resistance vectors.

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## REFERENCES

- Bedford ID, Briddon RW, Brown JK, Rossel RC, Markham PG. 1994. Geminivirus transmission and biological characterisation of *Bemisia tabaci* (Genn.) biotypes from different geographic regions. *Ann Appl Biol* 125: 311-325.
- Briddon RW, Patil BL, Bagewadi B, Nawaz-ul Rehman MS, Fauquet CM. 2010. Distinct evolutionary histories of the DNA-A and DNA-B components of bipartite begomoviruses. *BMC Evol Biol* 10: 97. DOI: 10.1186/1471-2148-10-97.
- Brown JK, Idris AM, Torrez-Jerez I, Banks GK, Wyatt SD. 2001. The core region of the coat protein gene is highly useful for establishing the provisional identification and classification of begomoviruses. *Arch Virol* 146: 1581-1598.
- Chellappan P, Vanitharani R, Fauquet CM. 2004. Short interfering RNA accumulation correlates with host recovery in DNA virus-infected hosts, and gene silencing targets specific viral sequences. *J Virol* 78: 7465-7477.
- Czoznek H, Hariton-Shalev A, Sobol I, Gorovits R, Ghanim M. 2017. The incredible journey of begomoviruses in their whitefly vector. *Viruses* 9: 1-19
- Daryono BS, Natsuaki KT. 2002. Application of random amplified DNA markers for detection of resistant cultivars of melon (*Cucumis melo* L.) against cucurbit virus. *Acta Horticulturae*. 588:321-329
- Eagle PA, Orozco BM, Hanley-Bowdoin L. 1994. A DNA sequence required for geminivirus replication also mediates transcriptional regulation. *Plant Cell* 6: 1157-1170.
- Fauquet CM, Stanley J. 2003. Geminivirus classification and nomenclature: Progress and problems. *Ann Appl Biol* 142: 165-189
- Garrido-Ramirez ER, Sudarshana MR, Gilbertson RL. 2000. Bean golden yellow mosaic virus from Chiapas, Mexico: Characterization, pseudorecombination with other bean-infecting geminiviruses and germplasm screening. *Phytopathology*. 90: 1224-1232.
- Hanley-Bowdoin L, Settlage SB, Orozco BM, Nagar S and Robertson D. 1999. Geminiviruses: Models for Plant DNA Replication, Transcription, and Cell Cycle Regulation. *Crit Rev Plant Sci* 18: 71-106.
- Harrison BD, Robinson DJ. 1999. Natural genomic and antigenic variation in whitefly-transmitted geminiviruses ( begomoviruses). *Ann Rev Phytopathol* 37: 369-398.
- Hernandez-Zepeda C, Idris AM, Carnevali G, Brown JK, Moreno-Valenzuela OA. 2007. Preliminary identification and coat protein gene phylogenetic relationships of begomoviruses associated with native flora and cultivated plants from the Yucatan Peninsula of Mexico. *Virus Genes* 25: 825-833
- Idris AM, Hiebert E, Bird J, Brown JK. 2003. Two newly described begomoviruses of *Macroptilium lathyroides* and common bean. *Phytopathology* 93: 774-783.
- Inoue-Nagata AK, Lima MF, Gilbertson RL. 2016. A review of geminivirus (*Begomovirus*) diseases in vegetables and other crops in Brazil: current status and approaches for management. *Horticultura Brasileira* 34: 8-18
- Islam W, Lin W, Islam SU, Arif M, Li X, Yang Y, Ding X, Zhenguo D, Wu Z. 2018. Genetic diversity of begomoviruses in Pakistan captured through a vector based survey. *Microbial Pathogenesis* 118: 91-97
- Julijantono I, Somowiyarjo S, Trisyono AY, Daryono BS. 2010. Disease incidence of melon left curl in East Java and Special Province of Yogyakarta. *Jurnal Perlindungan Tanaman Indonesia* 16 (2): 76-81. [Indonesian]

- Kenyon L, Tsai W, Shih S, Lee L. 2014. Emergence and diversity of begomoviruses infecting solanaceous crops in East and Southeast Asia. *Virus Research* 186: 104-13 Kirthi N, Maiya SP, Murthy MRN, Savitri HS. 2002. Evidence of recombination among the tomato leaf curl virus strains/species from Bangalore, India. *Arch Virol* 147: 255-272.
- Kon T, Hidayat SH, Hase S, Takahashi H, Ikegami M. 2006. The natural occurrence of two distinct begomoviruses associated with DNA $\gamma$  and a recombinant DNA in a tomato plant from Indonesia. *Phytopathology* 96 (5): 517-525.
- Mayo MA, Pringle CR. 1997. *Virus Taxonomy*. J General Virol 79: 649-657.
- Maruthi MN, Colvin J, Seal SE, Gibson G, Cooper J. 2002. Coadaptation between cassava mosaic geminivirus and their local vector population. *Virus Res* 86: 71-85.
- McGrath PF, Harrison BD. 1995. Transmission of tomato leaf curl geminiviruses by *Bemisia tabaci*: Effects of virus isolate and vector biotype. *Ann Appl Biol* 126: 307-316.
- Mizutani T, Daryono BS, Ikegami M, Natsuaki K. 2011. First Report of Tomato leaf curls New Delhi virus Infecting Cucumber in Central Java, Indonesia. *Plant Dis* 95 (11): 1485
- Moriones E, Navas-Castillo J. 2000. Tomato yellow leaf curl virus, an emerging virus complex causing epidemics worldwide. *Phytopathology* 71: 123-134.
- Pan H, Chu D, Yan W, Su Q, Liu B, Wang S, Wu Q, Xie W, Jiao X, Li R, Yang N, Yang X, Xu B, Brown JK, Zhou X, Zhang Y. 2012. Rapid spread of Tomato Yellow Leaf Curl Virus in China is aided differentially by two invasive whiteflies. *PLoS One* 7 (4): e34817. DOI: 10.1371/journal.pone.0034817.
- Poonam R, Kulshreshtha A, Hallan V. 2017. Genome Organization of begomoviruses. In: Saxena S, Tiwari AK (eds). *begomoviruses: Occurrence and Management in Asia and Africa*. Springer Nature, Singapore
- Pita JS, Fondong VN, Sangare A, Otim-Nape GW, Ogwal S, and Fauquet CM. 2001. Recombination, pseudorecombination and synergism of geminiviruses are determinant keys to the epidemic of the cassava mosaic disease in Uganda. *J General Virol* 82: 655-661.
- Ramos PL, Guevara-González RG, Peral R, Ascencio-Ibañez JT, Polston JE, Argüello-Astorga GR, Vega-Arreguín JC, Rivera-Bustamante RF. 2003. *Tomato mottle Taino virus* pseudorecombines with PYMV but not with ToMoV: Implications for the delimitation of *cis*- and *trans*-acting replication specificity determinants. *Arch Virol* 148: 1697-1712.
- Revell PA, Ha CV, Porchum SC, Vu MT, Dale JL. 2003. The complete nucleotide sequence of two distinct Geminiviruses infecting Cucurbits in Vietnam. *Arch Virol* 148: 1523-1541
- Rodriguez-Pardina PE, Zerbini FM, Ducasse DA. 2006. Genetic diversity of begomoviruses infecting soybean, bean, and associated weeds in Northwestern Argentina. *Fitopatología Brazil* 31 (4): 342-348
- Rojas MR, Gilbertson RL. 2008. Emerging plant viruses: a diversity of mechanisms and opportunities. In: Roossinck MJ (ed), *Plant Virus Evolution*. Springer-Verlag, Berlin.
- Roossinck MJ. 1997. Mechanisms of plant virus evolution. *Ann Rev Phytopathol* 35: 191-209.
- Rusli ES, Hidayat SH, Suseno R, Tjahjono B. 1999. Virus Gemini asal cabai: kisaran inang dan cara penularan Bulletin HPT. 11: 26-31. [Indonesian]
- Sakamoto, Kon T, Hidayat SH, Ito K, Hase S, Takahashi H, Ikegami M, 2005. begomoviruses associated with leaf curl disease of tomato in Java, Indonesia. *J Phytopathol* 153 (9): 562-566.
- Saunders K, Bedford ID, Stanley J. 2002. Adaptation of an autonomously-replicating nano virus-like DNA component associated with ageratum yellow vein disease from whitefly to leafhopper transmission. *J General Virol* 83: 907-913.
- Seal SE, vanden Bosch F, Jeger MJ. 2006. Factors influencing *Begomovirus* evolution and their increasing global significance: Implications for sustainable control. *Crit Rev Plant Sci* 25: 23-46.
- Sharma P, Rishi N, Malathi VG. 2005. Molecular cloning of coat protein gene an Indian cotton leaf curl virus (CLCuV-HS2) isolate and its phylogenetic with other members of *Geminiviridae*. *Virus Genes* 30: 85-91
- Sulandari S, Suseno R, Hidayat SH, Harjosudarmo J, Sosromarsono S. 2006. Deteksi dan kajian kisaran inang virus penyebab penyakit daun keriting kuning cabai. *Hayati* 13: 1-6. [Indonesian]
- Snehi SK, Purvia AS, Parihar SS, Gupta G, Singh V, Raj SK. 2017. Overview of *Begomovirus* genomic organization and its impact. *Intl J Curr Res* 9 (11): 61368-61380.
- Tsai WS, Shih SL, Green SK, Akkermans D, Jan FJ. 2006. Molecular characterization of a distinct tomato-infecting *Begomovirus* associated with yellow leaf curl diseased tomato in Lembang, Java Island of Indonesia. *Plant Dis* 90 (6): 831
- Tsai WS, Shih SL, SK Green, Lee LM, Luther GC, Ratulangi M, Sembel DT. 2009. Identification of a new *Begomovirus* associated with yellow leaf curl diseases of tomato and pepper in Sulawesi, Indonesia. *Plant Dis* 93 (3): 321
- Wilisiani F, Somowiyarjo S, Hartono S. 2014. Identifikasi molecular virus penyebab penyakit daun keriting isolat Bantul pada melon. *Jurnal Perlindungan Tanaman Indonesia*. 18 (1): 47-54. [Indonesian]