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Identity and Sequence Diversity of Begomovirus Associated with Yellow Leaf Curl Disease of Tomato in Indonesia

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Infection of tomato by Begomovirus is known to cause serious disease and yield losses. Samples of tomato plants showing typical symptoms of begomovirus infection were collected from eight locations in Java and Sumatra. Amplification of a putative AV1 gene was performed using AV1 specific primers for Geminivirus, total nucleic acid isolated from tomato samples exhibiting leaf curl disease as the template, and the PCR technique. Direct sequencing of PCR product was carried out, followed by nucleotide and predicted amino acid sequence analysis using the BLAST program. Positive results were obtained, the PCR amplification proved that diseased tomato samples collected from eight locations in Java and Sumatra were infected with Begomovirus. When nucleic acid and amino acid sequences of the eight isolates were compared to other begomovirus's sequences present in the GenBank it was found that the isolates determined in this research were Indonesian isolates of AYVV. Further phylogenetic analysis of eight Begomovirus isolates identified in this study indicated they belonged into two different clades. Results of this research also suggest that the existence of Begomovirus genetic diversity in various regions in Indonesia needs further investigation. Moreover, the prevalence of distinct Begomovirus species or isolates also need investigation.

Keywords: begomovirus, sequence analysis, tomato leaf curl virus

The family Geminiviridae is one of the largest group of plant viruses. The morphology of geminivirus particles is unique having a twin shape and a small size (H" 30 x 20 nm). They are characterized by a circular, single stranded, DNA genome which replicates in the host cell nucleus and is encapsulated in twin incomplete icosahedral particles. The family Geminiviridae is divided into four genera, i.e., *Mastrevirus*, *Curtovirus*, *Topocuvirus*, and *Begomovirus*, based on the viral genome structure, host range and type of insect vector (Van Rogenmortel *et al*. 2000). *Mastreviruses* and *Curtoviruses* have a monopartite genome and are transmitted by various leafhopper species, but infect monocotyledonous and dicotyledonous plants, respectively. The genus *Topocuvirus* is made up of the tomato pseudo-curly-top virus, which has a monopartite genome and are transmitted by treehopper species, and which infects dicotyledonous plants. Members of the genus *Begomovirus* have monopartite (one ~2.9 kb DNA) or bipartite (two ~2.6 kb DNAs referred to as "DNA-A" and "DNA-B") genome, and are transmitted by whiteflies (e.g. *Bemisia tabaci* Gennadius) and infect dicotyledonous plants (Harrison 1985).

Begomoviruses are considered to be emerging plant viruses, due to their increasing incidence and the severity of the diseases which they cause in a number of economically important crops, mostly in tropical and subtropical regions in the world (Polston and Anderson 1997). In Indonesia, begomoviruses are currently a spreading threat for cultivated tomatoes in some tomato production areas and causing substantial yield losses. These viruses have also been reported to infect some other plants such as chilli pepper (*Capsicum annuum*), ageratum (*Ageratum conyzoides*), and tobacco (*Nicotiana benthamiana*) (Sudiono *et al*. 2001).

Partial characterization of the genomic sequence of the Indonesian tomato-leaf-curl virus (ToLCIDV) was first reported in 1999 (DDBJ, accession number AF189018). Similar characterization was performed for six begomoviruses infecting tomato plants from Bandung, West Java (ToBadI-5, ToBadII-20, ToBadII-23, ToBadIII-1); Purwokerto, Central Java (ToPur-6); and Magelang, Central Java (ToMag-2) (Sukamto *et al*. 2005). Meanwhile, the complete nucleotide sequence identification has been reported for the ToLCIDV from Java (Kon *et al*. 2006).

In this paper, we report sequence analysis of the coat protein gene isolated from eight begomovirus isolates infecting tomato plants collected from different locations in Java and Sumatra. It is important to understand that the genetic diversity of begomoviruses infecting tomato plants provides basic information for developing disease control strategies.

MATERIALS AND METHODS

Sample Collection. Tomato plants showing typical symptoms of begomovirus infection (yellow mosaic, leaf curling, and stunting) were collected from several tomato producing areas (8 districts, 5 provinces) in Indonesia (Table 1). Samples were placed in plastic bags or bottles and carried to the laboratory for DNA extraction and to a screenhouse for virus isolation and propagation in host plant.

DNA Extraction and Polymerase Chain Reaction (PCR) Analysis. Total DNA was extracted from tomato leaves leaf according to Doyle and Doyle (1999) with slight

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Table 1 Isolate identity, observed symptoms on collected tomato samples, location of collected samples, and number of determined nucleic acid and predicted amino acid sequences based on the polymerase chain reaction amplified putative AV1 gene

Isolate identity	Observed symptoms on collected tomato sample	Location of collected sample	Size of sequences	
			Nucleotide (bp)	Amino acid (residues)*
ToLC-Blt	Leaf curling and stunting	Blitar, East Java	580	193
ToLC-Mlg	Yellowing, severe upward leaf curling, and stunting	Malang, East Java	529	176
ToLC-Srg	Leaf curling, stunting, and mosaic	Sragen, Central Java	685	227
ToLC-Mgl	Leaf curling, stunting, and smaller leaflet	Magelang, Central Java	707	235
ToLC-Byl	Leaf curling and stunting	Boyolali, Central Java	702	233
ToLC-Klu	Severe upward leaf curling, yellowing, and stunting	Kaliurang, D.I. Yogyakarta	605	201
ToLC-Bgr	Severe leaf curling, cupping, smaller leaf, and stunting	Bogor, West Java	666	221
ToLC-Btg	Leaf curling and stunting	Brastagi, North Sumatra	706	234

*Predicted based on the determined nucleotide sequences.

modification. Leaf tissue was ground in a sterile mortar in 1.0 ml of extraction buffer. The extraction buffer used for the initial homogenization contained 100 mM Tris pH 8.0, 1.4 M NaCl, 20 mM EDTA pH 8.0, and 0.2% (v/v) β-mercaptoethanol. The extraction buffer was autoclaved and 2% polyvinylpyrolidone (PVP) and 2% CTAB were added immediately before use. Immediately after grinding, 500 µl aliquotes were transferred to a 1.5 ml microfuge tube and incubated for 15 min at 65°C with occasional mixing to avoid aggregation of the homogenate. To the extract was added 500 µl of chloroform: isoamylalcohol (24:1.0) and the mixture was vortexed thoroughly. Each tube was then centrifuged for 15 min at 10 000 x g. The debris-free supernatant was then transferred to a new tube and proteins precipitated by adding 2.5 x volume of absolute ethanol and washed twice with 70% ethanol (v/v). The pellet was dried and resuspended in 100 µl of sterile distilled water. This DNA extract was stored at -20°C for further use.

The coat protein gene was amplified by the PCR technique using two oligonucleotide specific primers for the geminivirus coat protein gene that were provided by Dr. Sylvia Green from the Asian Vegetable Research Development Centre (AVRDC-Taiwan), i.e the CPPROTEIN-V1(5'-TAATTCTAGATGTCGAGCGA CCCGCCGA-3') and the CPPROTEIN-C1(5'-GGCCGA ATTCTTAATTTTGAACAGAATCA-3'). PCR reactions were prepared in 25 µl total volume, containing 10 x buffer (100 mM Tris-HCl, 500 mM KCl, pH 8.3), $MgCl_2$ (75 mM), dNTP mix (4 mM), 10 µM of each primer, 1 unit of Taq DNA polymerase, and 2 µl of the DNA template. The amplication profile consisted of 30 cycles of denaturation at 94°C for 1 min, primer annealing at 55°C for 1 min and primer extension at 72°C for 2 min, and followed by postextension at 72°C for 5 min. PCR products were analysed on agarose gels (1%) and stained with ethidium bromide and visualized under UV light using the Chemidoc gel system (Biorad).

Direct Sequencing of PCR Products. Products of the PCR were first visualized in agarose gels (1%) to estimate their concentration and to confirm their purity. Further purification of PCR products used ExoISAP digestion [Exonuclease I enzyme and Shrimp Alkaline Phosphatase/ SAP (Biorad)] to remove the excess primers and dNTPs. The purified PCR products were then diluted, and mixed with a single primer (either forward or reverse primer). Each sequencing reaction was prepared using a DTCS kit (Beckman Coulter) in a 20 µl volume containing 1.5 µM of either forward or reverse primer and 50 ng of template DNA. The reaction profile consisted of 45 cycles of denaturation at 96°C for 20 sec, primer annealing at 50°C for 20 sec, and primer extension at 60°C for 4 min. Reactions were analyzed in an CEQ 800 analyzer (Beckman Coulter).

Determination of Virus Identity. Database searches for the selected begomoviruses species were carried out using The National Center of Biotechnology Information basic local alignment search tool or NCBI BLAST(http://www.ncbi.nlm.nih.gov/BLAST) (Altschul *et al.* 1990). The identity of the virus was determined based on the highest percentage value of the AV1 gene nucleic acid and amino acid sequence among the evaluated isolates and available sequences in the GenBank DNA database. The sequences were aligned using ClustalW (Thompson *et al.* 1994) while phylogenetic analysis was conducted using online tool facilities available at http://www.genebee.msu.su/clustal/advanced.html. The distance matrices were calculated using the Kimura two-parameters model (Kimura 1980). Results of the analysis were used to construct phylogenetic tree and the robustness of the internal branches of the tree was tested by bootstrap analysis using 1 000 replicates.

RESULTS

The detection of begomovirus infection using specific primers for AV1 gene specific primers resulted in a single DNA fragment of approximately 780 bp (Fig 1) for most of the tomato plants collected from the eight locations which showed typical begomovirus symptoms (Fig 2). Since the oligonucleotide primers used for PCR were specific for amplifying coat protein (AV1) gene of Begomovirus, results of this research suggested the presence of Begomovirus in all of the tomato plants investigated. Direct sequencing of PCR products generated sequences of the putative AV1 gene ranging from 529-707 bp (Table 1). The determined nucleotide sequences was submitted to the GenBank Database. Homology among begomovirus isolates was shown when alignment was obtained for predicted amino acid sequence of partial AV1 gene of eight Begomovirus isolates identified in this research and other isolates available in the GenBank DNA database (Fig 3). Comparison of nucleotide and predicted amino sequences of putative AV1 gene of the eight isolates with available AV1 gene sequences in the GenBank revealed that the eight isolates had homologies above 90% with *Ageratum* yellow vein virus

isolate from Singapore (AYVV-GenBank acc. no. X74516) (Tan *et al*. 1995). The homology was less than 90% with a Pepper leaf curl virus isolate from Malaysia (PepLCV-Mal-AF414287) (Shih *et al*. 1998) or Cassava mosaic virus isolate from South Africa (CasMV-SA-AJ575560) (Table 2).

Distance matrices based on the AV1 gene amino acid sequences of suspected Begomovirus isolates examined in this research, AYVV, SCLV-Jpn, PepLCV-Mal, ToLCV-Jv, ToLCV-JvA, ToLCV-Mal, and SA-CasMV, supported previous findings that the suspected Begomoviruses were indeed isolates of AYVV since their distances (Table 3) were generally less than 10%. On the other hand, the distances were generally more than 10% if AV1 gene of the suspected Begomovirus isolates from Indonesia were compared with that of either PepLCV, ToLCV-Jv or ToLCV-JvA, and more than 20% when compared with that of CasMV-SA. These results indicated that the suspected Begomovirus isolates from Indonesia were not isolates of PepLCV, ToLCV-Jv, ToLCV-JvA or CasMV. The ToLCV-Jv and ToLCV-JvA were two other Begomovirus isolates from Indonesia that had previously been reported (Kon *et al.* 2006).

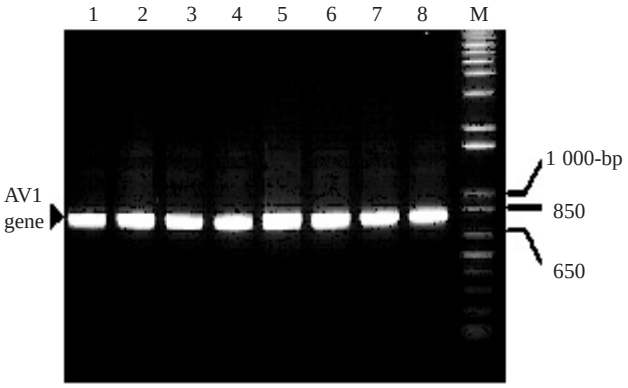

Fig 1 Agarose gel electropherogram of PCR amplified DNA fragments of putative AV1. The DNA fragments were amplified by PCR using AV1 specific primers and total nucleic acid of diseased tomato sample from (1) Malang, East Java; (2) Blitar, East Java; (3) Sragen, Central Java; (4) Magelang, Central Java; (5) Boyolali, Central Java; (6) Kaliurang, D.I. Yogyakarta; (7) Bogor, West Java; and (8) Brastagi, North Sumatera; M, 1 Kb plus (Invitrogen) DNA marker.

Phylogenetic analysis was carried out based on the predicted amino acid sequences of the putative AV1 gene determined in this research and those of other Begomoviruses available in the GenBank (Fig 4). The eight Begomovirus isolates determined in this research were all clustered in a similar clade with AYVV from Singapore (AYVV) and Taiwan (AYVV-Tw). However, their sequences were quite diverse based on the arm-length of the phyllogenetic tree. Results of this analysis also indicated that three Begomovirus isolates from Indonesia identified in previous study (ToLCV-Jv, ToLCV-JvA, and TYLCV-Lbg) did not belong to the same clade as the isolates identified in the present research. These three isolates were more closely related to PepLCV-Mal than to the eight isolates identified in the more recent research.

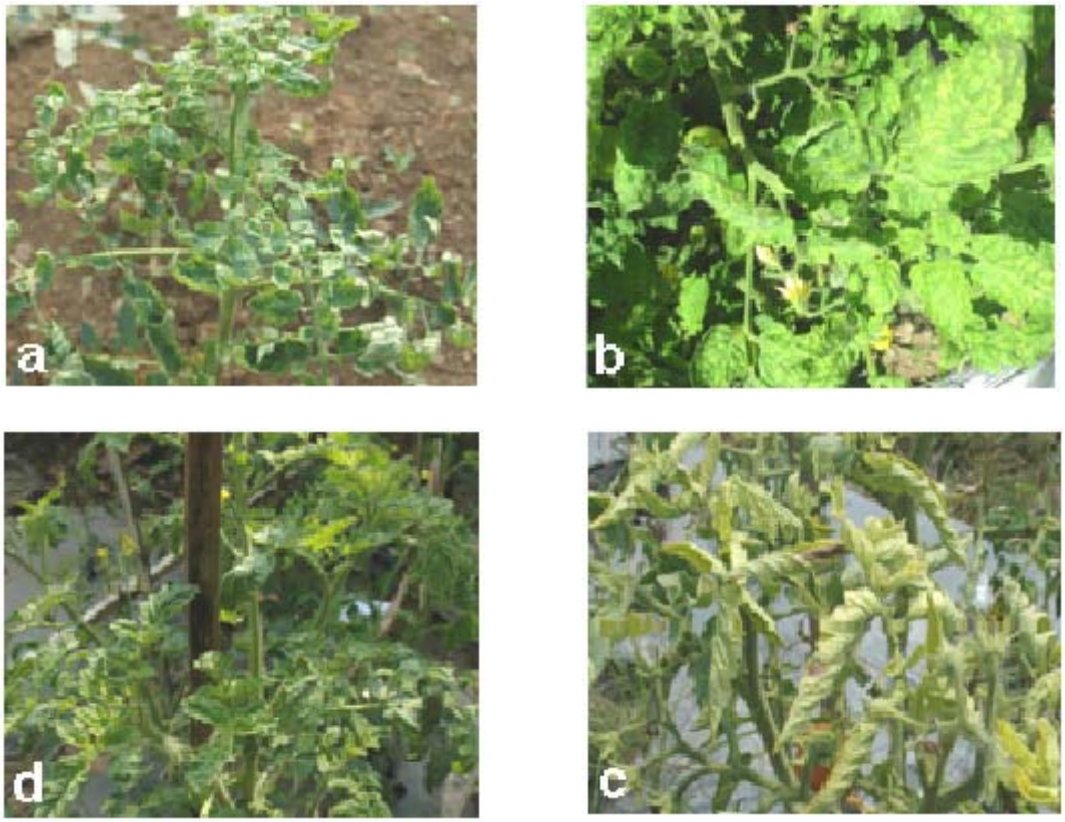

Fig 2 Tomato plants exhibited various leaf-curl symptoms. Subsequent experiment indicated they were infected by *Begomoviruses.* a, leaf curling, smaller leaflet, and stunting symptoms on tomato plant from Bogor, West java; b, leaf curling and mosaic symptoms on tomato plant from Sragen, Central Java; c, severe upward leaf curling and yellowing symptoms on tomato plant from Kaliurang, Di Yogyakarta; and d, leaf curling symptom on tomato plant from Blitar, East Java.

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ToLC-Btg        VLVTNKRRTWTNRPMYRKPRLYRMYRTPDVPKGCEGPCKVQSYEQRHDISHVGKVLCVSD
ToLC-Srg        VLVTNKRRTWTNRPMYRKPRLYRMYRTPDVPKGCEGPCKVQSYEQRHDISHVGKVLCVSD
ToLC-Bgr        VLVTNKRRTWTNRPMYRKPRLYRMYRTPDVPKGCEGPCKVQSYEQRHDISHVGKVLCVSD
ToLC-Mlg        VLVTNKRRTWTNRPMYRKPRLYRMYRSPDVPKGCEGPCKVQSYEQRHDISHVGKVLCVSD
ToLC-Blt        VLVTNKRRTWTNRPMYRKPRLYRMYRSPDVPKGCEGPCKVQSYEQRHDISHVGKVLCVSD
ToLC-Klu        VLVTNKRRTWTNRPMYRKPRLYRMYRSPDVPKGCEGPCKVQSYEQRHDISHVGKVCCVTD
ToLC-Byl        VFVTNKRRTWTNRPMYRKPSMYSMYRSPDVPKGCEGPCNVQSYEQRHDISHVGKVLCVSD
ToLC-Mgl        VLVTNKRRTWTNRPMYRKPRLYRMYRSPDVPKGCEGPCKVQSYEQRHDISHVGKVLCVSD
AYVV
SCLV-Jpn        VLVTNKRRTWTNRPMYRKPRMYRMYRSPDVPKGCEGPCKVQSYEQRHDISHVGKVLCVSD
ToLCV-Mal       VLVTNKRRAWTQRPMYRKPRMYRMYRSPDVPKGCEGPCKVQSYEQRHDISHVGKVLCVSD
ToLCV-JvA       VLVTNKRRTWTNRPMYRKPRLYRMYRSPDVPKGCEGPCKVQSFESRHDVSHVGKVCCITD
ToLCV-Jv        VLVTNKRRTWTNRPMYRKPRMYRMYRSPDVPKGCEGPCKVQSFESRHDVSHVGKVCCITD
PepLCV-Mal      VLVTNKRRSWANRPMNRKPRIYRMYKSPDVPRGCEGPCKVQSYEQRHDVAHVGKVICVSD
CasMV-SA        VQGTNKRRSWTLRPMYRKPRMYRMYRSPDVPRGCEGPCKVQSYEQRDDVKHTGAVRCVSD
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ToLC-Btg        VTRGNGLTHRVGKRFCVKSVYVLGKVWMDENIKTKNHTNTVMFYLVRDRRPYGT-AMDFG
ToLC-Srg        VTRGNGLTHRVGKRFCVKSVYVLGKVWMDGDIKTKNHTNTVMFYLVRDRRPYGT-ALDFG
ToLC-Bgr        VTRGNGLTHRVGKRFCVKSVYVLGKIWMDENIKTKNHTNTVMFYLVRDRRPYGT-AMDFG
ToLC-Mlg        VTRGNGLTHRVGKRFCVKSVYVLGKIWMDENIKTKNHTNTVMFYLVRDRRPYGT-AMDFR
ToLC-Blt        VTRGNGLTHRVGKRFCVKSVYMLGKIWMDENIKTKNHTNTVMFHLVRDRRPYGS-AMDFG
ToLC-Klu        VTRGNGLTHRMGKRFCVKSVYVLGKIWMDENIKTKNHTNTVMFYLVRDRRPYGS-AMDFG
ToLC-Byl        VTRGNGLTHRVGKRFCVRSVYVLGKIWMDENIKTKNHTNTVMFYLVRDRRPYGS-AMDFG
ToLC-Mgl        VTRGNGLTHRVGKRFCVKSVYVLGKIWMDENIKTKNHTNTVMFYLVRDRRPYGS-AMDFG
AYVV
SCLV-Jpn        VTRGNGLTHRVGKRFCVKSVYVLGKIWMDENIKTKNHTNTVMFYLVRDRRPFGT-AMDFG
ToLCV-Mal       VTRGNGFTHRVGKRFCVKSIYVLGKIWMDENIKTKNHTNTVMFFLVRDRRPFGT-AMDFG
ToLCV-JvA       VTRGLGLTHRTGKRFCVKSVYIMGKVWMDENIKTKNHTNTVMFFLVRDRRPYSS-PQDFG
ToLCV-Jv        VTRGLGLTHRTGKRFCVKSVYIMGKVWMDENIKTKNHTNTVMFFLVRDRRPYSS-PQDFG
PepLCV-Mal      VTRGNGLTHRVGKRFCIKSVYVLGKIWMDENIKTKNHTNTVMFFLVRDRRPFGT-PQDFG
CasMV-SA        VTRGSGITHRVGKRFCVKSIYVLGKIWMDENIKKQNHTNQVMFFLVRDRRPYGTSPMDFG
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ToLC-Btg        QVFNMYDNEPSTATIKNDLRDRYQVLRKFTSTVTGGQYACKEQAMV
ToLC-Srg        QVFNMYDNEPSTATIKNDLRDRYQVLRKFSSTVTGGQYACKRQAWV
ToLC-Bgr        QVFNMYDNEPSTATIKNDLRDRYQVLRKYTSTVTGGQYACKEQALV
ToLC-Mlg        QVFNMYDNEPSTATIKNDLRDRYQVLRKFTSTVTGGQYACKEQAWV
ToLC-Blt        QVFNMYDNEPSTATIKNDLRDRYQVLRKFTSTVTGGQYAAKEQASV
ToLC-Klu        QVFTMYDNEPSTATIKNDLRDRYQGVRKLSSTVTGGQYAGKGQASV
ToLC-Byl        QVFNMYDNEPSTATIKNDLRDRYQVLRKFTSTVTGGQYASKEQALV
ToLC-Mgl        QVYNMYDNEPSTATIKNDLRDRYQVLRKFTSTVTGGQYASKEQALV
AYVV
SCLV-Jpn        QVFNMYDNEPSTATIKNDLRDRYQVLRKFTSTVTGGQYACKEQALV
ToLCV-Mal       QVFNMYDNEPSTATVKNDMRDRYQVLRKFTATVTGGQYASKEQALV
ToLCV-JvA       QVFNMYDNEPSTATVKNDMRDRFQVLRKFSSTVTGGQYACKEQALV
ToLCV-Jv        QVFNMYDNEPSTATVKNDMRDRFQVLRKFTSTVTGGQYACKEQSLV
PepLCV-Mal      QVFNMYDNEPSTATVKNDNRDRFQVLRRFQATVTGGQYASKEQAIV
CasMV-SA        QVFNMFDNEPSTATIKNDLRDRFQVLRKFHATVVGGPSGMKEQALI
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Fig 3 Alignment of partial amino acid sequences predicted from determine nucleotide sequences of AV1 gene of eight Begomovirus isolates determined in this research and seven Begomovirus isolates available from GeneBank DNA database. AYVV-*Ageratum yellow vein virus* (X74516), SCLV-Jpn-*Soybean crinkle leaf virus-Japan* (AB050781), PepLCV-Mal-*Pepper leaf curl virus-Malaysia* (AF414287), ToLCV-Jv-*Tomato leaf curl virus-Java* (NC-005031), ToLCV-JvA-*Tomato leaf curl virus-Java* [*Ageratum*] (AB162141), ToLCV-Mal-*Tomato leaf curl virus-Malaysia* (NC-004648), and CasMV-SA-*South African cassava mosaic virus* (AJ575560) were obtained from GeneBank database at http://www.ncbi.nlm.nih.gov/blast/Blast.cgi.

Table 2 Percentages of nucleotide (NT) and amino acid (AA) sequence identities of AV1 gene among suspected Begomovirus isolates determined in this research and three Begomoviruses available in the GeneBank database

Isolate identity	AYVV		PepLCV-Mal		CasMV-SA	
	NT (%)	AA (%)	NT (%)	AA (%)	NT (%)	AA (%)
ToLC-Bgr	95	97	81	85	78	77
ToLC-Blt	93	95	81	85	89	77
ToLC-Btg	95	96	81	85	87	79
ToLC-Byl	92	93	80	83	86	78
ToLC-Klu	93	90	82	81	84	75
ToLC-Mgl	95	96	81	86	86	80
ToLC-Mlg	94	96	81	86	79	75
ToLC-Srg	94	93	82	81	82	76

AYVV-*Ageratum yellow vein virus* (X74516), PepLCV-Mal-*Pepper leaf curl virus-Malaysia* (AF414287), and CasMV-SA-*South African cassava mosaic virus* (AJ575560) were obtained from GeneBank database at http://www.ncbi.nlm.nih.gov/blast/Blast.cgi.

Table 3 Distance matrices (%) based on predicted AV1 gene amino acid sequences of suspected Begomovirus isolates determined in this research, *Ageratum yellow vein virus* (AYVV), *Soybean crinkle leaf virus* (SCLV), *Pepper leaf curl virus* (PepLCV), *Tomato leaf curl virus* (ToLCV), and C*assava mosaic virus* (CasMV)

Isolate	ToLC-Blt	ToLC-Mlg	ToLC-Srg	ToLC-Mgl	ToLC-Byl	ToLC-Klu	ToLC-Bgr	ToLC-Btg
ToLC-Blt								
ToLC-Mlg	3.7							
ToLC-Srg	7.7	5.0						
ToLC-Mgl	3.1	3.1	7.0					
ToLC-Byl	6.3	6.3	10.4	4.4				
ToLC-Klu	7.7	8.3	10.4	7.7	11.1			
ToLC-Bgr	4.4	2.5	5.0	3.1	6.3	8.3		
ToLC-Btg	4.4	2.5	3.7	3.7	7.0	9.0	1.8	
AYVV	5.0	3.7	6.3	3.1	6.3	9.7	2.5	3.1
SCLV-Jpn	4.4	2.5	6.3	3.1	5.0	9.0	2.5	3.1
PepLCV-Mal	16.2	15.5	18.5	15.5	18.5	20.9	16.2	16.2
ToLCV-Jv	14.0	14.7	15.5	14.0	17.7	15.5	14.7	14.0
ToLCV-JvA	14.7	15.5	17.7	14.7	17.0	17.7	15.5	14.7
ToLCV-Mal	9.0	8.3	12.5	7.7	9.7	14.7	8.3	9.0
CasMV-SA	22.5	23.3	27.6	21.7	23.3	27.6	23.3	24.2

AYVV-*Ageratum yellow vein virus* (X74516), SCLV-Jpn-*Soybean crinkle leaf virus-Japan* (AB050781), PepLCV-Mal-*Pepper leaf curl virus-Malaysia* (AF414287), ToLCV-Jv-*Tomato leaf curl virus-Java* (NC-005031), ToLCV-JvA-*Tomato leaf curl virus-Java* [*Ageratum*] (AB162141), ToLCV-Mal-*Tomato leaf curl virus-Malaysia* (NC-004648), and CasMV-SA-*South African cassava mosaic virus* (AJ575560) were obtained from GenBank database at http://www.ncbi.nlm.nih.gov/blast/Blast.cgi.

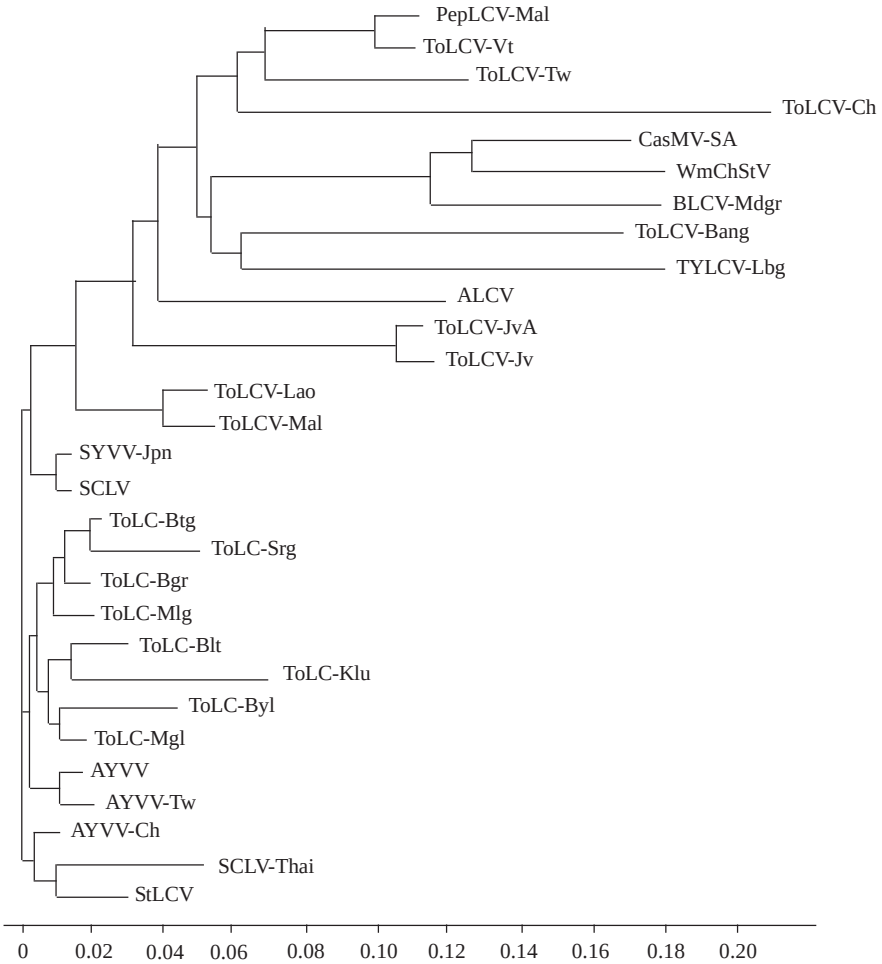

Fig 4 Phylogenetic relationship based on predicted AV1 gene amino acid sequences of suspected Begomovirus isolates determined in this research, and other Begomoviruses available in the GeneBank DNA database. The AV1 gene for AYVV-*Ageratum yellow vein virus* (X74516), AYVV-Tw-*Ageratum yellow vein Taiwan virus* (NC-004627), AYVV-Ch-*Ageratum yellow vein China virus*-[*G68*] (AJ849916), SCLV-*Soybean crinkle leaf virus* (AB050781), SLCV-Jpn-*Soybean crinkle leaf virus*-[*Japan*] (AB050781), SLCV-Thai-*Soybean crinkle leaf virus*-[*Thailand*] (EF064788), ToLCV-JvA-*Tomato leaf curl Java virus*-[*Ageratum*] (AB162141), TYLCV-Lbg-*Tomato yellow leaf curl Indonesia virus*-[*Lembang*] (AF189018), ToLCV-Jv-*Tomato leaf curl Java virus* (NC-005031), ToLCV-Bang-*Tomato leaf curl Bangladesh virus* (AF188481), ToLCV-Lao-*Tomato leaf curl Laos virus* (AF195782), ToLCV-Mal-*Tomato leaf curl Malaysia virus* (NC-004648), ToLCV-Vt-*Tomato leaf curl Vietnam virus* (NC-004153), ToLCV-Ch-*Tomato leaf curl China virus* (ToLCV-Ch), PepLCV-Mal-*Pepper leaf curl virus*-[*Malaysia*] (AF414287), StLCV-*Stachytarpheta leaf curl virus* (AJ810157), CasMV-SA-*South African cassava mosaic virus* (AJ575560), BLCV-Mdgr-*Bean leaf curl Madagascar virus* (AM701757), and WmChStV-*Watermelon chlorotic stunt virus* (NC-003708) isolates, respectively, were obtained from GeneBank DNA database, available at http://www.ncbi.nlm.nih.gov/blast/Blast.cgi.

DISCUSSION

A high incidence of leaf curl disease in tomato plants in Indonesia has been observed in the last 5 years and it has become a major problem in tomatoes growing in areas across the country (Hidayat *et al.* 2006). Association of begomiviruses with tomato leaf curl disease has been reported mainly from West Java and Central Java (Sudiono *et al*. 2001; Aidawati *et al*. 2005). Detailed analyses of the molecular properties and biological activities of begomoviruses from tomato plants with leaf curl in Java has been described recently (Sukamto *et al*. 2005; Kon *et al*. 2006). In this paper, we reported the detection, sequencing, and phylogenetic analysis of several isolates of tomato begomoviruses collected from different locations in Java and Sumatra, Indonesia. We conducted analysis of the genetic diversity based on coat-protein gene sequence after direct sequencing of PCR products. Direct sequencing of PCR products, after the PCR parameters were optimimized, has an advantage compared with other strategies, i.e. it is extremely efficient for the analysis of a large number of sequences in a short period of time.

Previously it has been known that several begomoviruses are associated with tomato leaf curl disease in Java, Indonesia. Based on sequence comparisons and phylogenetic analysis, the viruses were divided into several groups. It is an interesting fact that all begomoviruses asociated with tomato leaf curl disease in Java formed separate groups from those of other tomato infecting begomoviruses. According to Sukamto *et al*. (2005) and Kon *et al*. (2006), tomato begomoviruses from Java had a closest relationship with AYVV. Similarly, Begomovirus isolates identified in this research showed high sequence identities with that of AYVV, SCLV, and also ToLCV-Mal. The AV1 gene predicted amino acid sequences of the identified isolates exhibited distances of less than 10% against that of the three Begomoviruses, indicating they were isolates of the same virus species. Therefore, it was suggested that the identified Begomovirus isolates in this study might be Indonesian isolates of AYVV or SCLV. Based on AV1 gene sequences analysis in this study, previously identified tomato begomoviruses from Indonesia, ToLCV-Jv, ToLCV-JvA, and TYLCV-Lbg, had close relationships with PepLCV and CasMV. It was not the case for the eight identified Begomovirus isolates in this study since their predicted amino acid sequence identities and their distances were either more than 90% and less than 10% (against PepLCV) or more than 80% and less than 20% (against CasMV), respectively.

Although the eight Begomovirus isolates identified in this study exhibited more than 90% of the AV1 gene amino acid sequence identities and less than 10% of the distances, results of phylogenetic analysis indicated they belonged into two different clades. Such results indicated the their AV1 gene might have originated from the same progenitor sequences but separated a different way because of accumulated mutations. Another possible explanation might be through recombination. Differences in accumulated mutations might not be the answer since the occurence of Begomovirus-associated-tomato-diseases in Indonesia is very recent. Therefore, recombination might be the possible cause of such differentiation. More studies would be required before such a possibility could be decided. Kitamura *et al*. (2004) has proposed that recombination is a very frequent event and widespread phenomenon among Geminiviruses. Such recombination might occur at both species and genera levels. It was also suggested that the process of genome recombination within Geminiviruses contributed significantly to the evolution of Geminiviruses.

Based on the analysis above, it is suggested that the existence of Begomovirus genetic diversity in various regions in Indonesia needs further investigation. Moreover, the prevalence of distinct Begomovirus species or isolates should also be investigated. Such knowledge will aid the development of control strategies for viruses and support the development of Begomovirus resistant tomato cultivars through plant breeding.

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