Multiple infections of Begomovirus on its host plants

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Abstract. Begomovirus infection has been reported to cause many important diseases on vegetable crops in Indonesia. This research was conducted to reconfirm the identity of Begomovirus infecting several host plants. Plant samples were taken from several DI Yogyakarta locations, i.e., Sleman, Kulon Progo, and Bantul. Amplification of virus target was carried out by PCR method using three primer pairs, i.e., SPG1/2, PepYLCIVA-F/A-R, and PepYLCIV B-F/B-R. Begomovirus DNA fragments were successfully amplified from all samples using SPG1/2 primers, which confirmed the presence of Begomovirus infection in chili, eggplant, cucumber, and weed plants. The nucleotide sequences analysis identified five species of Begomovirus, i.e., PYLCIV, ToLCNDV, LUYVV, TYLCIV, and TYLCKaV; each has homology with sequences in GenBank 86.76% -98.12%, 96.11%, 94.36%, 95.48%, 90%-99.29%, respectively. Sequence analysis of DNA fragments amplified using PepYLCIV B-F/B-R specific primers confirms that the genome type of PYLCIV is bipartite. Furthermore, the data showed a double infection of PYLCIV with other Begomovirus species, i.e., with TYLCKaV in chili and eggplant, with TYLCIV and LUYVV in weeds, and with ToLCNDV in cucumbers. A double infection or a mixture of several viruses can cause recombination, potentially leading to new virus strains.

1. Introduction

Disease epidemics caused by Begomovirus have been widely reported in Indonesia. Leaf curl disease on tobacco plants in East Java, which is caused by TLCV (Tobacco leaf curl virus), was reported to cause yield losses of up to 30% [1]. Infection of PYLCV (Pepper yellow leaf curl virus) was first reported on chili plants in West Java, causing yellow leaf curl disease [2]. Since then, the disease was reported to widely spread in various chili cultivation areas in Java [3], Aceh [4], West Sumatra and Lampung [5], Central Java and Yogyakarta [6], Bali [7], West Nusa Tenggara [8], and Sulawesi [9]. Similarly with Begomovirus infection on tomato plants (Tomato yellow leaf curl virus/TYLCV), eggplant (Tomato yellow leaf curl/TYLC-Kanchanaburi virus), yard long beans (Mung bean yellow mosaic/MYM-India virus) and also in ageratum weeds (Ageratum yellow vein virus/AYVV) [10][11][12][13].

Yellow leaf curl disease always appears in every growing season. This is due to a disease inoculum source in the field, given the Begomovirus's extensive host range [14]. Furthermore, mixed infection of Begomovirus, i.e., TYLCKaV, PepYLCIV, and AYVV, was reported on chili plants in North Sumatra [4]. This paper discusses the identification of various Begomovirus infecting main horticultural crops in Yogyakarta.
2. Materials and methods

2.1. Plant samples collection
Plant samples were taken from several locations in Yogyakarta (Bantul, Sleman, Kulonprogo), which are known as Begomovirus endemic areas. Three planting fields were selected; plant samples were taken from each field consisting of chili, eggplant, cucumber, and weeds. Plant samples taken showed typical Begomovirus infection symptoms, involving yellowing and curling leaves, reduced leaf size, and leaf malformation in chili, eggplant, cucumber, or yellowing and vein clearing on weeds. The number of plant samples ranged from 1 to 7, depending on the variety of symptoms found.

Molecular identification of field samples was carried out at the Plant Virology Laboratory, Plant Protection Department, Faculty of Agriculture, IPB and CelTech Laboratory, Department of Integrative Biotechnology, Sungkyunkwan University, South Korea.

2.2. Amplification of target Begomovirus DNA by polymerase chain reaction (PCR) method
Total plant DNA extraction from 100 mg of leaf samples was carried out using CTAB buffer [100 mM Tris HCl (pH 8), 4 mM EDTA (pH 8), 1.4 M NaCl, 2% CTAB, 1% PVP, 0.5% 2-mercaptoethanol] and a mixture of chloroform: isoamyl alcohol (24: 1) as described by [15]. DNA precipitation was carried out using 3 M sodium acetate (pH 5.2) and isopropanol; the formed DNA pellets were then washed with ethanol (70%); after the DNA pellet was air-drying, it was dissolved in 50 µl of nuclease-free water.

DNA amplification of the target virus was performed using the PCR method. The amplification reaction consisted of 1 µl of DNA template, 1 µl of each primer with a concentration of 1 µM, 10 µl of the reagent mixture from DreamTaq Green amplification kit (Thermo Scientific, US) or Accu Power PCR Master Mix (BioNeer, Korea) and distilled water to make up 20 µl final reaction. Amplification was carried out using a thermocycler (Gene Amp. PCR System 9700 PE Applied Biosystem) with a preheating stage at 94°C for 5 min, followed by 35 amplification cycles consisting of separating the DNA strands at 94°C for 1 min, attaching the primer to the DNA template at 50°C for 1 min and DNA synthesis at 72°C for 1 min; the last cycle ends at 72°C for 1 min, and the reaction is stored at 4.0°C.

Begomovirus amplification was performed using three pairs of primers (Table 1). SPG1/SPG2 primer pairs were used for all test samples. Confirmation of PepYLCIV infection was carried out using primer pairs PepYLCIV A-F/PepYLCIV A-R and PepYLCIV B-F/PepYLCIV B-R to amplify DNA-A and DNA-B, with annealing temperatures at 63°C and 58°C, respectively (Table 1). Visualization of the amplified DNA product was carried out using electrophoresis on 1% agarose gel. Purification of amplified DNA using the MG PCR Purification Kit (MG Med, Korea) proceeded before sequencing.

2.3. DNA sequencing and analysis
The purified DNA of the amplified product was used as the sample for sequencing. The sequencing results were then analyzed first using the BLAST program available at NCBI (http://www.ncbi.nlm.nih.gov/BLAST/), followed by CLUSTAL_X and Mega 7 programs.
Table 1. Primers used to amplify Begomovirus

| Primer code | Nucleotides sequence (5’ – 3’) | PCR product (bp) | Source |
|-------------|--------------------------------|-----------------|--------|
| SPG1        | CCCCKGTGCWGWARATCCAT           | 912             | [13]   |
| SPG2        | ATCCVAAYWYACGAGGAGCT           |                 |        |
| PepYLCIV A-F | ACAGCAACTATCAAGAAGACGATC      | 468             | [14]   |
| PepYLCIV A-R | ATCTGGAATCGTTTACGTCCTC       |                 |        |
| PepYLCIV B-F | TGTCTCTCATCGTAGTCACACA       | 385             | [14]   |
| PepYLCIV B-R | GAAGATAGTCTGAGCGTCAT          |                 |        |

3. Result

Begomovirus fragments were successfully amplified from all samples using universal primers SPG1/2, which amplified part of the replication gene (AC1 gene) and part of the transcription activator protein gene (AC2 gene). These results confirmed the infection of Begomovirus on chili, eggplant, cucumber, and weed plants. Begomovirus infection causes diverse symptoms, with the primary symptom being yellow, curly leaves with the leaf surface curling upwards (Fig 1 and Table 2). The nucleotide sequence analysis of the amplified fragments identified five Begomovirus species, namely PYLCIV, Tomato leaf curl New Delhi virus (ToLCNDV), Ludwigia yellow vein Vietnam virus (LUYVVV), Tomato yellow leaf curl Indonesia virus (TYLCIV), and Tomato yellow leaf curl Kanchanaburi virus (TYLCKaV) each has homology with the sequence in GenBank of 86.76% -98.12%, 96.11%, 94.36%, 95.48%, 90% -99.29%, respectively (Table 2). PYLCIV infection in chilies, ToLCNDV in cucumber, and TYLCKaV in eggplant have been previously reported [16-17, 12]. Infection of LUYVVV has never been reported in Indonesia, so this is the first report of LUYVVV infection on Ludwigia peruviana weed. Another new information obtained from this study is the infection of Begomovirus species in host plants that have never been previously reported, i.e., TYLCIV on weeds, PYLCIV on eggplant, cucumber, and weeds.

![Figure 1](image1.jpg)

**Figure 1.** Infection of Begomovirus causing leaf yellowing on chili (a), eggplant (b), cucumber (c), and weed Ludwigia spp. (d)

Furthermore, the results showed multiple infections of PYLCIV with other Begomovirus species, i.e., with TYLCKaV in chilies and eggplant, with TYLCIV and LUYVVV in weeds, and with ToLCNDV in cucumbers (Table 3). Multiple infections or a mixture of several viruses may induce recombination, which will potentially produce new virus strains [18]. The incidence of interspecies recombination, mutation, and genetic drift has been reported earlier in TYLCSV (Tomato yellow leaf curl Sardinia virus), although the effect on the biology of the virus has not been confirmed [19].
Table 2. Disease symptoms in several host plants and identity of Begomovirus isolate from DI Yogyakarta (Bantul, Sleman, Kulon Progo Regency)

| Virus isolate | Isolate code | Plant host | Symptoms<sup>a</sup> | Sequence homology |
|---------------|--------------|------------|----------------------|-------------------|
| Bantul        | BC2          | Chili      | VB, LC, Y            | PYLCIV            |
| Bantul        | BC7          | Chili      | VB                   | TYLCKaV           |
| Bantul        | BC8          | Chili      | LC, R, WL            | PYLCIV            |
| Bantul        | GH2          | Eggplant   | VB, Y                | TYLCKaV           |
| Sleman        | G2           | Weed       | Y                    | TYLCIV            |
| Sleman        | G3           | Ludwigia   | CL, VB               | LUYVVV            |
| Sleman        | SC1          | Chili      | VB, LC, Y            | PYLCIV            |
| Sleman        | SC2          | Chili      | VB                   | PYLCIV            |
| Sleman        | SC7          | Chili      | LS                   | PYLCIV            |
| Sleman        | Tim1         | Cucumber   | VB                   | ToLCNDV           |
| Kulon Progo   | TR1          | Eggplant   | VB, Y                | TYLCKaV           |
| Kulon Progo   | C1KP         | Chili      | VB                   | PYLCIV            |
| Kulon Progo   | C2KP         | Chili      | LC, R, WL            | PYLCIV            |

<sup>a</sup> Symptoms: LC, leaf curling; R, reduced leaf area; VB, vein banding; WL, white leaf edge; Y, yellowing; Sg, stunted growth; C, cupping; N, necrosis; Cl, chlorosis; LS, wide leaf surface

<sup>b</sup> Begomovirus species: PYLCIV, Pepper yellow leaf curl Indonesia virus; TYLCKaV, Tomato yellow leaf curl Kanchanaburi virus; LUYVVV, Ludwigia yellow vein Vietnam virus; ToLCNDV, Tomato leaf curl New Delhi virus

Chili yellow curly leaf disease caused by PYLCIV is an essential disease in chili plants in Indonesia. Since it was first reported in 1999 in West Java [16], the disease has rapidly spread to all chili plantations in Indonesia. A study on the characterization of PYLCIV isolates from West Java [16], Yogyakarta [20], and West Sumatra [21] only reported the presence of the DNA-A molecule. The DNA-B molecule was successfully detected by [22] from Begomovirus infecting chili, tomato,
and weeds (*Ageratum* spp.) in Bandung, West Java. Our study again confirmed the DNA-B molecule from all samples from Yogyakarta using PYLCIV DNA-B specific primers. Sequence analysis of DNA fragments showed a homology of 94% - 98.29% to DNA-B PYLCIV from Bandung (Genbank no. AB267835.1). Thus, it can be concluded that the isolate of PYLCIV from Yogyakarta has a bipartite genome.

**Table 3.** Multiple infections of Begomovirus in several plants in Bantul, Sleman, and Kulon Progo Regency, DI Yogyakarta

| Field location and plant host | Isolate code | PYLCIV | TYLCKaV | TYLCIV | TolCNDV | LUYVVV |
|------------------------------|--------------|--------|---------|--------|---------|--------|
| **Bantul**                   |              |        |         |        |         |        |
| Chili                        | BC2          | +      | -       | -      | -       | -      |
| Chili                        | BC7          | +      | +       | -      | -       | -      |
| Chili                        | BC8          | +      | -       | -      | -       | -      |
| Eggplant                     | GH2          | +      | -       | -      | -       | -      |
| **Sleman**                   |              |        |         |        |         |        |
| Weed                         | G2           | +      | -       | +      | -       | -      |
| Ludwigia                     | G3           | +      | -       | -      | -       | +      |
| Chili                        | SC1          | +      | -       | -      | -       | -      |
| Chili                        | SC2          | +      | -       | -      | -       | -      |
| Chili                        | SC7          | +      | -       | -      | -       | -      |
| Cucumber                     | Tim1         | +      | -       | -      | +       | -      |
| **Kulonprogo**               |              |        |         |        |         |        |
| Eggplant                     | TR1          | +      | +       | -      | -       | -      |
| Chili                        | C1KP         | +      | -       | -      | -       | -      |
| Chili                        | C2KP         | +      | -       | -      | -       | -      |

+ and - indicates virus-positive and negative by PCR, respectively

4. **Conclusion**

Infection of five Begomovirus species, i.e., *Pepper yellow leaf curl Indonesia virus* (PYLCIV), *Tomato leaf curl New Delhi virus* (ToLCNDV), *Ludwigia yellow vein Vietnam virus* (LUYVVV), *Tomato yellow leaf curl Indonesia virus* (TYLCIV), and *Tomato yellow leaf curl Kanchanaburi virus* (TYLCKaV) were successfully detected from chili, eggplant, cucumber and weed plants in the Yogyakarta area. PYLCIV is the dominant species and was found to infect singly or mixed with other species. It was further confirmed that PYLCIV has a bipartite genome.

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