

Tomato Leaf Curl New Delhi Virus Associated with Yellow Mosaic Disease of Cucumber (*Cucumis sativus*) in Bengkulu, Indonesia

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ABSTRACT

Cucumber mosaic virus, Papaya ringspot virus, Squash mosaic virus, Cucurbit aphid-borne yellows virus, Cucurbit aphid-borne yellows virus Tobacco mosaic virus, Tomato yellow leaf curl New Delhi virus and Zucchini yellow mosaic virus were among the viruses that infected cucumbers in Java, according to a serology test. A virus from the Begomovirus group and the widely occurring viruses causes severe illnesses in cucumbers. The yellow mosaic disease is caused by Begomovirus, which affects Solanaceae, Cucurbitaceae, Leguminosae, and various weeds. Begomovirus has been found in several horticultural crops in Indonesia's diverse areas. Tomato yellow leaf curl New Delhi virus, Squash leaf curl Philippines virus, Squash leaf curl Philippines virus, and Pepper yellow leaf curl Indonesia virus are three Cucurbit-infecting begomoviruses that have been reported in Java Island. Begomovirus infection in cucumbers, on the other hand, has never been reported in Bengkulu. A survey of cucumber farming sites in Bengkulu observed some plants with yellow mosaic and vein banding symptoms. This study aims to use polymerase chain reaction (PCR) to identify Begomovirus-infected cucumber in Bengkulu utilizing SPG1/SPG2 as universal primers for Begomovirus. Cucumber leaf samples with the yellow mosaic and vein banding symptoms confirmed as Begomovirus infection. Begomovirus infecting cucumber plants in Bengkulu shows the highest homology (96.04 percent) with Tomato leaf curl New Delhi Virus (ToLCNDV) isolate from *Cucumis sativus* in Northern Sumatera (Indonesia) with accession number LC511775, according to nucleotide sequencing. Tomato leaf curls New Delhi Virus infection on cucumber was first reported in Bengkulu. The information on virus identification is critical and can be used as a foundation for controlling yellow mosaic disease on cucumbers.

Keywords: *Begomovirus, Cucumber, Homology, Yellow mosaic*

1. INTRODUCTION

Cucumber (*Cucumis sativus* L.) is a Cucurbitaceae vegetable with a wide range of adaptations. In Indonesia, cucumbers are grown in every region, including Sumatra, Java, Kalimantan, Sulawesi, Bali, Nusa Tenggara, Maluku, and Papua. Cucumber plants are primarily grown on Java Island among these places. Cucumbers in

Java were shown to be infected by seven viruses in prior investigations, including Cucumber mosaic virus, Cucurbit aphid-borne yellows virus, Papaya ringspot virus, Squash mosaic virus, Tobacco mosaic virus, Tomato yellow leaf curl New Delhi virus and Zucchini yellow mosaic virus. Virus frequency varied based on sample locations and cultivars in the fields [1], and virus dissemination was widespread in Java.

Begomoviruses produce a variety of crop diseases in both temperate and tropical climates. Begomovirus has caused severe yield losses in Indonesia, particularly Cucurbitaceae and Solanaceae. Squash leaf curl Philippines virus [2] is one of three Cucurbit-infecting begomoviruses found on Java Island. Squash leaf curl China-virus (SLCCNV), ToLCNDV, and Ageratum yellow vein virus (AYVV) were the three Begomovirus species linked to cucumber yellowing illness [3]. Begomovirus infection in cucumbers, on the other hand, has never been reported in Bengkulu.

Begomovirus with twinned isohedral particles encasing monopartite or bipartite circular single-stranded DNA genomes. Each of their genomes is around 2600 nucleotides long [4]. Leaf distortion and stunting, as well as some combination of golden-light and green-yellow mosaic/mottle, leaf curling, crumpling, veinal or interveinal yellowing, and yellow dots in leaves, are all indications of Begomovirus infection [5]. Discovered yellow mosaic symptoms on cucumber leave in Bengkulu. This study aims to use polymerase chain reaction (PCR) and universal primers for Begomovirus to identify Begomovirus-infected cucumbers in Bengkulu. The information on virus identification is critical and can be used as a foundation for controlling yellow mosaic disease on cucumbers.

This knowledge is critical for designing effective control measures to prevent the virus from spreading to areas that are not currently present.

2. MATERIAL AND METHODS

2.1. DNA Virus Detection by Polymerase Chain Reaction

2.1.1. Total DNA Extraction

We used Doyle's [6] technique to identify DNA virus with slight modifications to extract total DNA from symptomatic leaves. Liquid nitrogen was used to grind leaf tissue in a sterile mortar. Then mixed the leaf powder was with 500 µl of extraction buffer. 100 mM Tris pH 8.0, 1.4 mM NaCl, 20 mM EDTA pH 8 and 1% (v/v) -mercaptoethanol were used in the extraction buffer. Before using, the extraction buffer was autoclaved and immediately added 2 percent (v/v) polyvinyl pyrrolidone (PVP) and CTAB. To avoid homogenate aggregation, 500 µl aliquots were transferred to a 2 ml microtube after grinding and incubated for 60 minutes at 65 °C with mixing. The extract was mixed with 500 µl of chloroform: isoamyl alcohol (24:1) and vortexed extensively. The microtube was then centrifuged for 15 minutes at 13 000 rpm in a Biosan microspine. The supernatant was then transferred to a fresh tube. Protein was precipitated by adding 2/3 x volume of isopropanol to the supernatant centrifuged for 10 minutes at 12 000 rpm in the micro spine (Biosan). The pellet was rinsed in

70% ethanol (v/v) and centrifuged for 5 minutes at 8 000 rpm in the Biosan microspine. The pellet was dried before being resuspended in 100 µl of nuclease-free water. For future usage, the whole DNA extract was kept at -20 °C.

2.1.2. DNA Amplification

The genomes of Begomovirus amplified by PCR using a universal primer SPG1 (5'-CCCCKGTGCGWRAATCCAT-3') and SPG2 (5'-ATCCVAAAYWTYCAGGGAGCTAA-3') for transcriptional activator protein (TrAp) and replication-associated protein (Rep) genes [7]. Go taq green master mix (2x) (Thermo Fisher Scientific, Waltham, MA, USA), 10 mM per primer, 1µl DNA template, and ddH₂O was used to make PCR reactions in a total volume of 25 µl. The amplification was carried out in SimpliAmp thermal cycles, starting with a pre-heating step of 5 minutes at 94 °C, then followed by 30 cycles of denaturation (1 minute at 94 °C), annealing (1 minute at 59 °C), and extension (1 minute at 72 °C). After the last cycle, the temperature was raised to 72 °C for 10 minutes before being lowered to 4 °C.

2.1.3. DNA Visualization on agarose gel by electrophoresis

Amplification products were separated on a 1% agarose gel in 0.5X TBE buffer with nucleic acid staining with Ethidium bromide at 50 volts for 50 minutes. The PCR results were seen using agarose gel electrophoresis. Furthermore, the agarose gel visualization was saved on a gel documentation (Axygen), and the picture results were saved on a computer.

2.2. DNA Analysis

The amplification product is processed for nucleotide sequencing in FirstBASE Laboratories (Malaysia). To establish the identity of the sample, sequence analysis nucleotides begin with an alignment step that compares the sample to other Begomovirus nucleotides in GenBank. The Bio Edit program version 7.05 (<http://mbio.ncsu.edu/BioEdit/bioedit.html>) was used to analyze genetic diversity. The homology analysis uses information from GenBank, such as isolate sequences from other Begomoviruses from various parts of Indonesia and other countries. Cluster software was used to perform nucleotide sequence homology analysis (www.ebi.ac.uk). ClustalX, software Bio Edit version 7.05, and MEGA 6.0 program with neighbor-joining technique and bootstrap 1000 times were used to create a phylogenetic tree.

3. RESULTS

Based on visual symptoms, infected plants were found in the field. Yellow mosaic and vein banding on cucumber leaves have been seen in the area (Figure 1). Viruses from one Cucurbitaceae plant may usually infect



Figure 1. Symptom yellow mosaic and vein banding disease on cucumber leaves.

other Cucurbitaceae plants. Cucumber is thought to be infected by viruses that have been documented to infect other Cucurbitaceae. Researchers discovered signs on Cucumber in Bali comparable to those of a viral infection, such as chlorosis and mottle leaf deformity, and vein banding, which are indicators of a plant virus infection. Viruses are transferred between plants by vector insects.

The polymerase chain reaction method was used to detect and identify the virus, with universal primers for Begomovirus, namely SPG1/SPG2. DNA fragment 912 bp was successfully amplified from leaf samples (Figure 2). This confirmed that Begomovirus had infected cucumber leaf samples with yellow mosaic and vein banding symptoms. In Bali, Begomovirus infection in cucumbers was also recorded [8].

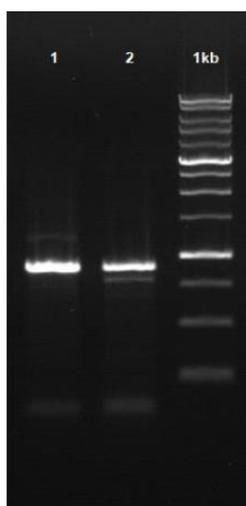


Figure 2. Electrophoresis of PCR product for Begomovirus in symptomatic leaves using 1% agarose gel stained with ethidium bromide. PCR products: (1-2), DNA samples; (1kb) marker 1 kb DNA ladder

The amplicon was sequenced then analyzed using a primary local alignment search method. Begomovirus infecting cucumber plants in Bengkulu shows the highest homology (96.04 percent) with Tomato leaf curl New Delhi Virus (ToLCNDV) isolate from *Cucumis sativus* in Northern Sumatera (Indonesia) with accession number LC511775, according to nucleotide sequencing. The first report on Cucumber cucumber infection by the New Delhi Virus in Bengkulu.

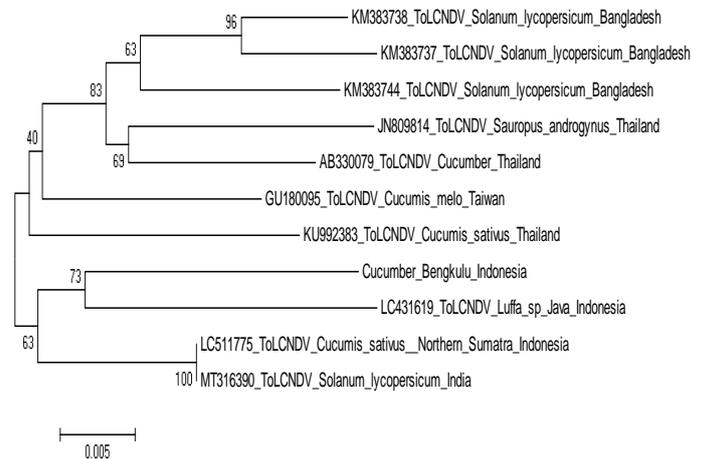


Figure 3. Phylogenetic tree of Tomato leaf curl New Delhi Virus (ToLCNDV) isolate from cucumber in Bengkulu with ToLCNDV from *GeneBank*.

Analysis of phylogeny was performed by comparing sample sequences to other Begomovirus sequences in GenBank (Figure 3). Cucumber samples from Bengkulu have a sequence identity of 95.04-96.04 percent with different ToLCNDV strains in Genebank. ToLCNDV infecting plants in Bangladesh, Thailand, Taiwan, India, and Indonesia provided comparative sequences.

Infection with several types of viruses in Cucurbitaceae plants, particularly viruses in cucumbers and melons, has been linked to several diseases. Tomato leaf curl New Delhi virus, Squash mosaic virus, zucchini yellow mosaic virus, and Cucumber mosaic virus are the causes of yellow, curly, and blistering mosaics on cucumbers in West Java, Central Java, and Yogyakarta Special Region [9]. Cucurbit aphid-borne yellow mosaic virus (CABYV) [10] and Tobacco mosaic virus (TMV) [1] are two novel viruses that have been discovered in cucumber plants in various parts of Java. Tomato leaf curls New Delhi Virus infection on cucumber was first reported in Bengkulu.

Tomato leaf curl New Delhi virus (ToLCNDV) is one member of begomovirus (genus Begomovirus, family Geminiviridae) that spread by the whitefly *Bemisia tabaci*. ToLCNDV causes 100% yield loss in tomato crops [11]. After the first occurrence of tomato leaf curl disease (ToLCD) in India [12], 48 species have been identified as ToLCD-related viruses. ToLCNDV is a typical virus that causes ToLCD and was first discovered in India in 1994 [13]. This virus has spread to Bangladesh

and Pakistan on the Indian subcontinent and Indonesia, the Philippines, Sri Lanka, Taiwan, and Thailand in Southeast Asia and Iran in the Middle East [12].

For many years, begomoviruses could not be spread by virus-infected seeds and had to rely on whitefly *B.tabaci* as vector, grafting, and artificial inoculation with infectious clones as their only means of transmission. According to recent research, the begomoviruses sweet potato leaf curl virus, mung bean yellow mosaic virus, tomato yellow leaf curl virus, bitter melon yellow mosaic virus, and Dolichos yellow mosaic virus have seed-transmissible properties [14]. Seed transmission of the ToLCNDV Indian strain has also been discovered in Chayote, India [15]. ToLCNDV has posed a severe danger to several cucurbit crops in Indonesia. The new information on virus identification is critical and can be used to reduce cucumber yellow mosaic disease. To minimize future yield loss in Cucurbitaceae plants, a study into ToLCNDV infection is required.

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