

SHORT COMMUNICATION

Identification of Polerovirus Infecting Chili Pepper in Java

Identifikasi Polerovirus yang Menginfeksi Tanaman Cabai di Jawa

Andri Saputra, Giyanto, Sari Nurulita, Sri Hendrastuti Hidayat*

Department of Plant Protection, Faculty of Agriculture, IPB University
Jalan Kamper, Kampus IPB Dramaga, Bogor, Jawa Barat 16680

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ABSTRACT

Infection of polerovirus in chili peppers has been reported from various parts of the world, including Indonesia. Pepper vein yellows virus (PeVYV), a member of genus *Polerovirus* were reported infecting chili plants in Bali, Java, and Aceh during 2012 to 2020. Recently, symptoms of the polerovirus infection, such as yellowing leaves with vein thickening, were found in chili pepper crops in the Central Java Region, specifically in Brebes, Magelang, and Kulon Progo. Molecular detection by RT-PCR method using universal polerovirus primers O3R/PL4F, followed by sequence analysis, confirmed the presence of PeVYV on samples from Magelang and Kulon Progo. Furthermore, the isolates of PeVYV are identified as PeVYV-2, PeVYV-6, and PeVYV-8. These current incidences indicate the expansion of the polerovirus distribution in Indonesia.

Keywords: pepper vein yellows virus, RT-PCR, sequence analysis, vein thickening

ABSTRAK

Infeksi polerovirus pada tanaman cabai semakin banyak dilaporkan dari berbagai bagian dunia termasuk di Indonesia. Infeksi pepper vein yellows virus (PeVYV) pada tanaman cabai di Bali, Jawa, dan Aceh telah dilaporkan pada kurun waktu 2012 sampai 2020. Belum lama ini gejala infeksi polerovirus berupa daun kuning dengan penebalan tulang daun ditemukan pada pertanaman cabai di wilayah Jawa Tengah, yaitu di Brebes, Magelang dan Kulon Progo. Deteksi molekuler dengan metode RT-PCR menggunakan primer universal polerovirus O3R/PL4F yang dilanjutkan dengan analisis sekuens membuktikan adanya infeksi PeVYV dari sampel asal Magelang dan Kulon Progo. Identifikasi lebih lanjut menunjukkan bahwa isolat PeVYV tersebut ialah PeVYV-2, PeVYV-6, dan PeVYV-8. Insidensi infeksi PeVYV ini menunjukkan perluasan distribusi polerovirus di Indonesia.

Kata kunci: analisis sekuens, pepper vein yellows virus, penebalan tulang daun, RT-PCR

*Corresponding author: Department of Plant Protection, Faculty of Agriculture, IPB University. Jalan Kamper, Kampus IPB Dramaga, Bogor, Jawa Barat 16680.
Phone: +62 251 8629364, Fax: +62 251 8629362, Email: srihendrastuti@apps.ipb.ac.id.

Chili pepper is one of important horticultural commodities globally, including in Indonesia due to its high economic value. Pests and diseases have been known to cause problems in chili pepper production. Viral diseases have been reported to cause significant yield loss. Virus infections in chili pepper plants can result in a variety of symptoms ranging from mild to severe mosaic, wrinkled and curled leaves, chlorosis, and even stunted growth. Several viruses have been reported to infect chili pepper plants in Indonesia including tobacco mosaic virus (TMV), chili veinal mottle virus (ChiVMV), cucumber mosaic virus (CMV), potato virus y (PVY), pepper vein yellows virus (PeVYV), pepper mild mottle virus (PMMoV), and pepper yellow leaf curl virus (PYLCV) (Suastika *et al.* 2012; Damiri 2014; Selangga *et al.* 2021; Damayanti and Kurniawati 2022).

Member of genus *Polerovirus* has been known to infect chili pepper worldwide. The polerovirus species that has been reported to infect chilies in the world is PeVYV. Infection of polerovirus on chili pepper causing yellowing and rolling of leaves was reported by Murakami and Kawano (2017) in Japan. Chlorosis between leaf veins and shortened internodes were reported by Dombrovsky *et al.* (2010) on chili pepper plants in Israel. Furthermore, Tomassoli *et al.* (2016) reported yellowish leaf spot on chili pepper plant infected by polerovirus in Italy. Infections by the PeVYV on chili peppers in Indonesia were reported in Bali in 2012 (Suastika *et al.*

2012) and Aceh in 2020 (Koeda *et al.* 2020). Knierim *et al.* (2013) also found and reported polerovirus infection on chili pepper plants in Rembang, Central Java, which showing green mosaic and chlorosis of the leaves.

A survey conducted in mid-2023 in several chili pepper fields in the Central Java Region, including Brebes, Magelang, and Kulon Progo, revealed dominant viral infection symptoms, such as yellow mosaic, whitish yellow mosaic, and green mosaic. Based on previous reports, symptoms associated with polerovirus involve green mosaic with thickening of leaf veins and chlorosis. Following PCR detection using universal Begomovirus primers (SPG1/SPG2) followed by sequencing, a total of eight samples were confirmed positive for PYLCV (data not shown). In a number of chili pepper fields, symptoms of leaf chlorosis with slight thickening of leaf veins resembling polerovirus infection symptoms were also observed (Figure 1).

Detection of polerovirus was carried out using reverse transcription polymerase chain reaction (RT-PCR) method with universal polerovirus primers (PL4F – 5' TGC GAC AAA TAG TTA ATG AAT ACG GT 3' and O3R – 5' GTC TAC CTA TTT BGG RTT NTG GAA 3'), which amplify the coat protein region (Corrêa *et al.* 2005). Total RNA extraction from chili pepper leaf samples was performed using a GeneJET RNA Purification Kit (Thermo-Fisher Scientific, Waltham, US). The amplification process consisted of cDNA synthesis at 45 °C for 60 minutes, followed by pre-denaturation

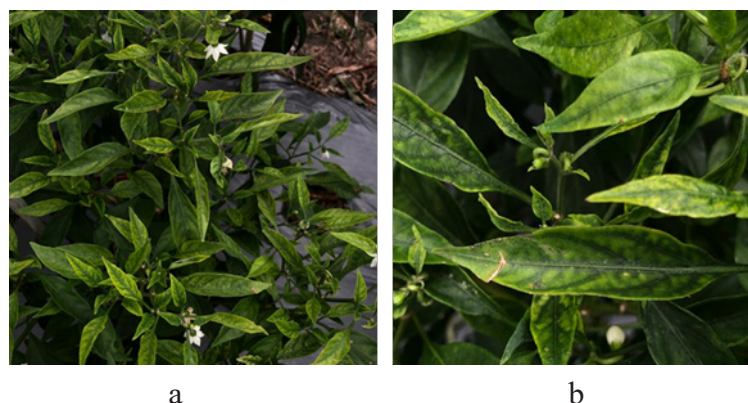


Figure 1 Disease symptoms in chili pepper plants in Central Java associated with polerovirus infection. a, chlorosis and leaf reduction; and b, chlorosis with slight thickening of leaf veins.

at 95 °C for 1 minute, and then 35 cycles consisting of denaturation at 95 °C for 30 seconds, annealing at 50 °C for 30 seconds, elongation at 72 °C for 1 minute and one cycle of post-extension at 72 °C for 10 minutes. A specific DNA fragment of 650 bp was successfully amplified from chili pepper plant samples from Magelang and Kulon Progo (Figure 2). The amplified products were transported for purification and sequencing to First Base, Malaysia. The obtained sequencing data were then compared to sequence databases

from GenBank and analyzed using the BioEdit V.7.0.5 software and MEGA 11 application. Based on the sequence analysis, the PeVYV, member of genus *Polerovirus* was identified in the chili pepper samples from Magelang and Kulon Progo. Furthermore, it was found that the homology level of PeVYV from chili pepper plants in Central Java ranged from 88.18% to 96.76% compared to the GenBank data (Figure 3). Three PeVYV sequences from chili pepper plants in Central Java have been submitted to GenBank with accession numbers

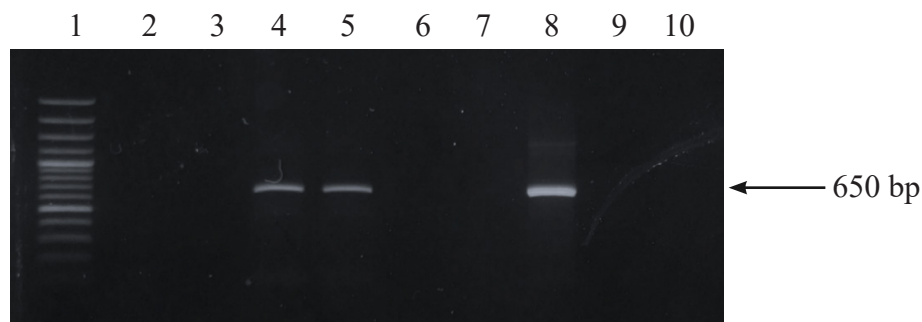


Figure 2 Visualization of DNA fragments resulting from amplification using universal polerovirus primers O3R/PL4F with a target amplicon of 650 bp. Lane 1: 100 bp DNA marker (Thermo-Fisher Scientific); lanes 2 and 3: samples from Brebes; lanes 4 and 5: samples from Magelang; lanes 6, 7, 8, and 9: samples from Kulon Progo; and lane 10: ddH₂O as the negative control.

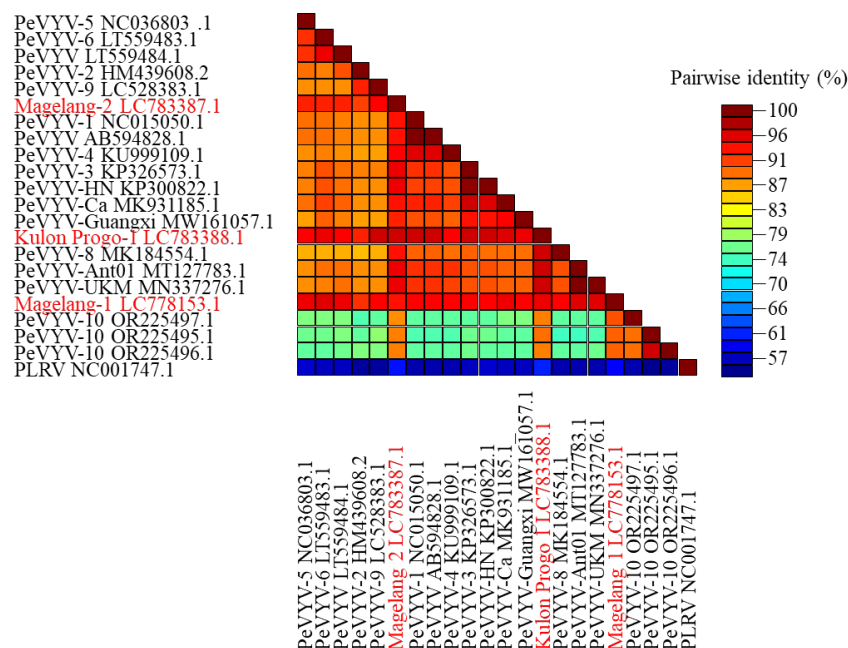


Figure 3 Pairwise identity matrix of *Pepper vein yellows virus* (PeVYV) inferred using SDT matrix in 'two-color' mode. The isolates of PeVYV from Java are indicated by red letter. The percentage of pairwise identity is visualized by the color-coded boxes. *Potato leaf roll virus* (PLRV) was used as an outgroup.

LC778153.1, LC783387.1, and LC783388.1. Subsequently, a phylogenetic tree analysis was conducted using MEGA 11. Three groups were formed in the phylogenetic tree: Magelang 1 isolate (LC778153.1) clustered together with PeVYV-6 LT559483.1 (90.59%); Magelang 2 isolate (LC783387.1) was closely related to PeVYV-2 HM439608.1 (93.33%); while Kulon Progo 1 isolate (LC783388.1) grouped with PeVYV-8 MK184554.1 (96.11%) (Figure 4). Therefore, it is concluded that chili pepper plants showing symptoms of chlorosis with slight leaf vein thickening are associated with polerovirus infection. PeVYV-2 and PeVYV-6 can be said to be new discoveries and it is possible that there are still other PeVYV species that infect chili plants in

Indonesia. The host range of polerovirus is known to be very wide, including cotton, cucurbits, corn, melons, chilies, potatoes, sugar cane, and wheat (Knierim *et al.* 2014; Agrofoglio *et al.* 2017; Gonçalves *et al.* 2017). There are 26 species of polerovirus and generally transmission occurs persistently by aphid vectors and can spread viruses from both the vegetative and generative phases (Bello *et al.* 2021; La Tourette *et al.* 2021). Polerovirus infection in Indonesia has been reported in cucumber plants (Adnyani 2018; Laili and Damayanti 2019; Kurnia *et al.* 2022), in addition to chili pepper plants (Suastika *et al.* 2012; Knierim *et al.* 2013; Koeda *et al.* 2020). Considering the development of polerovirus infections in the world, including in Indonesia

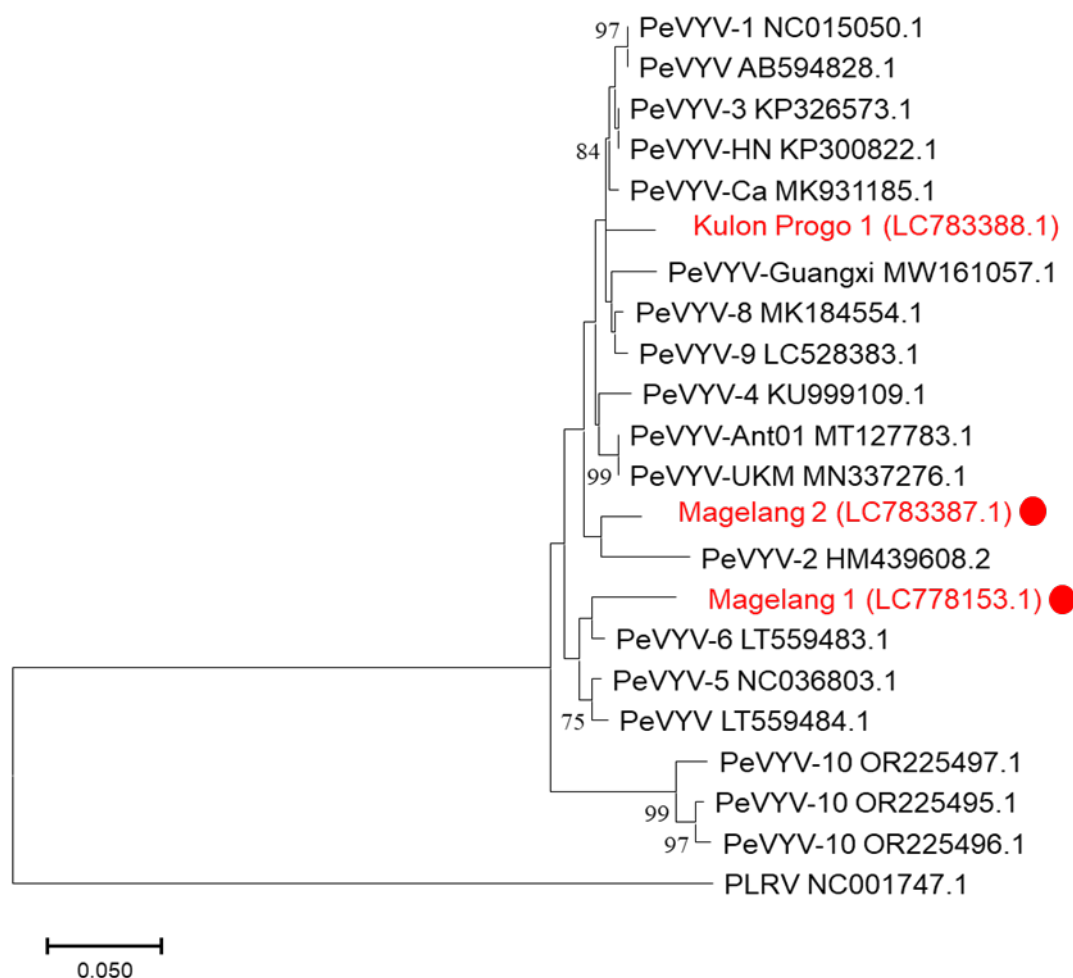


Figure 4 A phylogenetic tree shows the relationship between *Pepper vein yellows virus* (PeVYV) isolates from Java and isolates from other countries. This phylogenetic tree was constructed using the Maximum-Likelihood method in MEGA 11. The PeVYV isolates from this study consist of Magelang 1 (LC778153.1), Magelang 2 (LC783387.1), and Kulon Progo 1 (LC783388.1). The PLRV NC001747.1 was used as outgroup.

which is increasingly widespread, efforts are needed to inhibit it. Identifying polerovirus to the species level and studying its virulence can support strategies to control the diseases it causes.

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