Jurnal Perlindungan Tanaman Indonesia, Vol. 29, No. 1, 2025: 55−61
DOI: 10.22146/jpti.99401
Available online at http://jurnal.ugm.ac.id/jpti
ISSN 1410-1637 (print), ISSN 2548-4788 (online)

Research Article

Molecular Diversity of Pepper yellow leaf curl Indonesia virus (PepYLCIV) the Cause of Yellow Leaf Curl Disease in Chili Pepper (*Capsicum frutescens*) in Tuban Regency, East Java, Indonesia

Nurenik[1)]* & Adi Rastono[1)]

[1)]*Horticulture Cultivation Study Program, Politeknik Pertanian dan Peternakan Mapena*
Jln. Imam Bonjol, Podang, Lajo Lor, Singgahan, Tuban Regency, East Java, 62361 Indonesia

**Corresponding author. E-mail: nurenik.djapan@gmail.com*

Received August 28, 2024; revised October 16, 2024; accepted July 7, 2025

ABSTRACT

Yellow leaf curl disease caused by *Begomovirus* is a significant threat to chili peppers in Indonesia. This disease has been widespread at several chili pepper production centers in Java, Sumatra, Bali, Lombok, and Sulawesi. Tuban Regency is one of the chili peppers (*Capsicum frutescens* L.) production centers in East Java, but research on *Begomovirus* infection is still limited. The study aimed to molecularly characterize and identify *Begomovirus* that infect chili pepper plants in Tuban. The research method includes symptom observation in the field in four subdistricts in Tuban, molecular detection via PCR, and sequences analysis. Types of symptoms found in chili pepper plants in Tuban were yellowing, curling, curving, green mosaic, leaf cupping upward and downward, and reduction in leaf size. All samples from Grabagan, Bancar, Soko, and Jatirogo Subdistricts were infected by *Begomovirus* based on the AV1 gene (coat protein) using PCR techniques with target 580 bp DNA fragments. Sequence homology results proved that the pepper yellow leaf curl Indonesia virus (PepYLCIV) of the genus *Begomovirus* infected chili pepper plants in Tuban. The phylogenetic tree demonstrated that all isolates from Tuban (PQ187395 - PQ187398) were closely related and within the same group as the PepYLCIV isolates from Ngablak Subdistrict, Magelang Regency, Central Java, Indonesia (OP846605). These isolates from Tuban formed a separate group from PepYLCV Malaysian isolates (MW389931).

Keywords: *Begomovirus*; chili pepper; disease; PepYLCIV

INTRODUCTION

Yellow leaf curl disease caused by *Begomovirus* is a major threat to horticultural crops, such as chili pepper, tomato, sweet potato, and ornamental plants (Kandito *et al*., 2021; Selangga & Listihani, 2021). This disease is one of the main factors inhibiting chili cultivation worldwide, which could harm plants and result in a yield loss of 20−100% (Nalla *et al*., 2023; Widodo *et al*., 2023). Most cultivated chili peppers cultivars in Indonesia are susceptible to yellow leaf curl disease caused by pepper yellow leaf curl Indonesia virus (PepYLCIV), belonging to the *Begomovirus* genus (Cania *et al*., 2021; Sayekti *et al*., 2021; Ayu *et al*., 2021).

In Indonesia, pepper-yellow diseases have been known since the 2000 (Sulandari, 2006; Fadhila *et al*., 2020). The incidences of yellow leaf curl disease caused by PepYLCV have been reported in chili pepper plants from various regions in Indonesia, including Java (Wahyono *et al*., 2023; Santosa *et al*., 2024), Sumatra (Kesumawati *et al*., 2019), Bali (Selangga & Listihani, 2021; Temaja *et al*., 2024); Sulawesi (Widodo *et al*., 2023), and Lombok Island (Windarningsih, 2019).

The insect vector, *Bemisia tabaci*, facilitates the transmission of PepYLCV in the field. It can persistently spread the virus meaning once it feeds on a virus-loaded plant, it will persist in the insect's body throughout its lifespan (Nigam, 2021; Temaja *et al*., 2022; Nalla *et al*., 2023). In addition, PepYLCIV has been confirmed as a seed-transmissible virus in chili pepper plants, and viruses can be transmitted from seeds to new seedlings (Fadhila *et al*., 2020). PYLCV infection in chili pepper plants causes various symptoms

such as yellowing, curling, mosaics, curving, bending upward or downward, and stunting (Ayu *et al*., 2021; Selangga & Listihani, 2021; Temaja *et al*., 2024). All symptoms that appeared are due to the disruption of nutrition flow (photosynthate) from source to sink because the virus in the plant invades the phloem (Folimonova & Tilsner, 2018).

Current PepYLCV management is ineffective because the virus often mutates (Cania *et al*., 2021). Moreover, the association between *Begomovirus* and DNA satellites can affect the symptoms severity (Kandito *et al*., 2021). Genome editing to obtain genetic resistance through plant breeding is one solution to manage the PepYLCV. For that purpose, information on the genome sequence of the PepYLCV is necessary (Rubio *et al*., 2020).

Research on *Begomovirus* infection on chili pepper has been reported in Java by Wahyono *et al*. (2023), including regions in Central Java (Sragen, Banjarnegara, Wonosobo, and Karanganyar), West Java (Sukabumi, Garut, and Bandung Barat), and East Java (Kediri, Banyuwangi, Malang, Batu, and Magetan). East Java has large production area of chili pepper. In 2022, Banyuwangi Regency produced up to 1,042,988 quintals; Banyuwangi 1,042,988 quintals; Malang up to 874,337 quintals, Kediri up to 811,942 quintals; Sampang up to 491,112 quintals; Blitar up to 446,746 quintals; and Tuban up to 366,783 quintals of chili pepper (*Badan Pusat Statistik Provinsi Jawa Timur*, 2023). Although Tuban is one of the main chili pepper production centers, research on *Begomovirus* infection in this region is limited. Therefore, this study aimed to identify the *Begomovirus* types infecting chili pepper plants in Tuban and characterize PepYLCV molecular features. Moreover, this research contributes significantly to understanding the distribution and phylogeny of PepYLCV in Indonesia.

MATERIALS AND METHODS

Field Survey and Samples Collection

A survey was conducted in four subdistricts. Sample collections in Jatirogo and Bancar Subdistricts were carried out on April 27, 2024, while in Soko and Grabagan Subdistricts was carried out on May 5, 2024. From each location, leaves were taken from five plants with typical symptoms of *Begomovirus*, and composited into 1 sample per subdistrict, a total of 4 samples were tested using PCR.

DNA Extraction

Leaf composite samples were taken from each area to identify *Begomovirus* species. A hundred milligrams of fresh sample was used for total DNA extraction. Extraction was done by following manufacturer instructions using a total DNA extraction kit for plants (Geneaid, Germany). Total genomic DNA was subsequently used as a template for amplification.

Detection and Amplification Using PCR

Polymerase Chain Reaction (PCR) reactions were carried out using a final volume of 50 µL, consisting of 25 µL MyTaq Redmix Polymerase (Bioline), 19 µL ddH_2O, 2 µL forward primer (10 µM), 2 µL reverse primer (10 µM), and 2 µL DNA templates. The primer sequences Krusty/Hommer used in this study are listed in Table 1. Detection of *Begomovirus* was performed by amplifying the coat protein (AV1) gene, partial cds. This gene is widely used as a diagnostic marker due to its conserved nature across *Begomovirus* species. The thermal cycler program was predenaturation at 95 °C for 3 min, followed by 35 cycles of denaturation at 95 °C for 1 min, annealing at 55 °C for 30 s, and extension at 72 °C for 30 s. The final extension was at 72 °C for 10 min. Visualization of PCR products was carried out by agarose gel electrophoresis 1% (w/v) using 50 V power for 50 min.

Table 1. Universal primers used for *Begomovirus* detection.

Primer	Sequence (5' - 3')	Size (bp)	References
Krusty (Forward)	5'-CCNMRDGGHTGTGARGGNCC-3'	580	Revill *et al*., 2003
Hommer (Reverse)	5'-SVDGCRTGVGTRCANGCCAT-3'		

Sequencing and Sequence Analysis

PCR products were purified and subjected to direct Sanger sequencing using both forward and reverse primers. The sequencing was performed by PT Genetika Science, Jakarta. Nucleotide sequence results were assembled and edited using BioEdit, then analyzed using the BLAST tool from NCBI to compare sequence similarity (homology) with known *Begomovirus* isolates available in GenBank. Multiple sequence alignment was performed using ClustalW, and phylogenetic analysis was carried out in MEGA X program using the Maximum Likelihood method with 1,000 bootstrap replications to assess the robustness of the phylogenetic tree.

RESULTS AND DISCUSSION

Disease Symptoms

Samples were collected from chili pepper plants in four subdistricts (Bancar, Soko, Jatirogo, and Grabagan) from 4 farmer's fields in Tuban, which showed typical symptoms of *Begomovirus* infections and various symptoms were observed, including yellowing, curling, curving, green mosaic, leaf cupping upward and downward, and reduced leaf sizes (Figure 1). These symptoms had also been reported as typical of yellow leaf curl disease of chili pepper in Java Island, Indonesia (Wahyono *et al.*, 2023). Some of the symptoms of *Begomovirus* in chili plants, such as dwarfing with leaf yellowing and malformation, have been previously reported in various regions in Indonesia, including Bali (Temaja *et al.*, 2024).

The diversity of symptoms caused by *Begomovirus* in chili pepper can be influenced by virus strain, plant genotype, the age of the plant when infected, environmental conditions, and vector activity (Gaswanto *et al.*, 2016; Rubio *et al.*, 2020). Although Begomovirus infection does not kill the chili pepper plant, fruit productions were reduced (Figure 1A and 1D). Sayekti *et al.* (2021) reported that fruit weight per chili pepper plant infected by *Begomovirus* generally decreased when compared to healthy plants. In severe symptoms, leaf size is reduced, flowers fall out and do not produce fruit (Figure 1C).

Fadhila *et al.* (2020) have reported that PepYLCIV is seed-transmissible in chili pepper plants. Interviews with farmers revealed that they did not know that the virus can be transmitted through seeds.

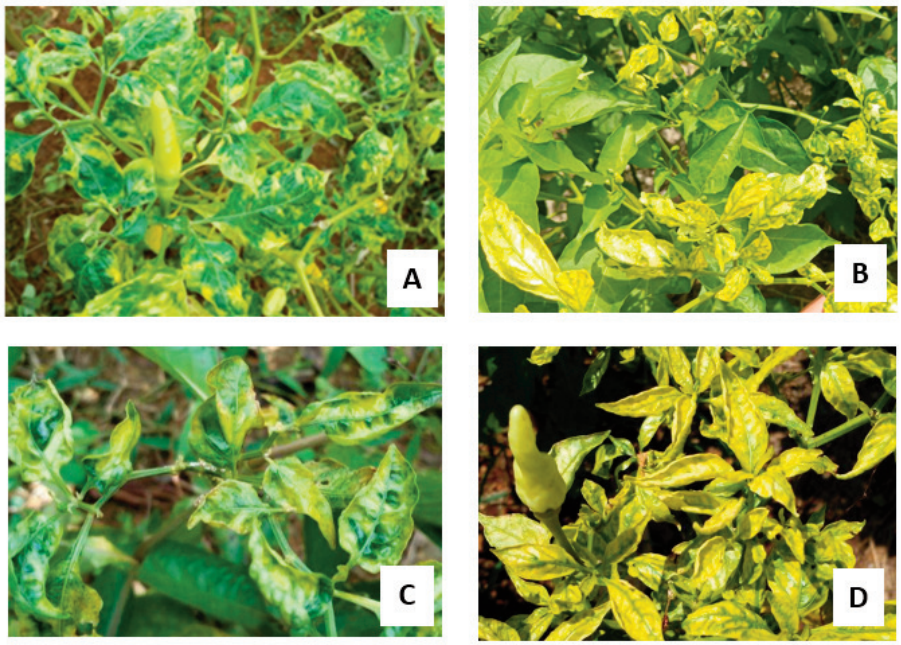

Figure 1. Symptoms variation of chili pepper plants infected by PepYLCIV verified by a PCR detection. (A) Green mosaic and curving in Jatirogo-Tuban; (B) yellowing, curling,and curving in Soko-Tuban; (C) yellowing, curling, curving, and leaf of reduced size in Bancar-Tuban; (D) yellowing, leaf cupping upward and downward in Grabagan-Tuban.

Bad chili cultivation practiced by farmers in Tuban, such as using seeds from infected plants harvested in the previous season, and seedlings on open area has become one of triggers for the development of early inoculum. Therefore, further emphasis should be focused on healthy seedling preparation for outbreak prevention. Nevertheless, Tuban's chili cultivation is often intercropped with corn plants to control *Bemisia tabaci* populations. Yolanda *et al.* (2024) explained that volatile compounds produced by corn were able to prevent *B. tabaci* infestation on chili crops.

Molecular Identification of *Begomovirus* in Tuban

Result of polymerase chain reaction (PCR) with a pair of *Begomovirus* universal primers Krusty & Homer (Revill *et al.*, 2003) showed that symptomatic leaf samples on chili pepper plants in four subdistricts of Tuban Regency (Jatirogo, Soko, Bancar, and Grabagan) were infected with the virus. The 580 bp amplified fragment was similar to results from chili plant samples in Central Java (Santosa et al., 2024) and Southeast Sulawesi (Widodo *et al.*, 2023).

Sanger sequencing analysis showed that the *Begomovirus* from chili pepper samples collected from Tuban, East Java, Indonesia, was included in pepper yellow leaf curl Indonesia virus (PepYLCIV) group. PepYLCIV Bancar, Soko, Jatirogo, and Grabagan isolates have a degree of genetic similarity (homology) of 88.8 to 96.3% with twelve PepYLCIV groups (Table 2), which has been previously reported in the GenBank. PepYLCIV nucleotide sequence homology from the four subdistricts proves that the PepYLCIV of the genus *Begomovirus* infected chili pepper plants in Tuban, East Java.

The phylogenetic tree (Figure 2) demonstrated that Grabagan (PQ187395), Jatirogo (PQ187398), Bancar (PQ187396), and Soko (PQ187397) isolates were closely related and formed a group with the PePYLCIV isolates from Ngablak Subdistrict, Magelang Regency, Central Java, Indonesia (OP846605). This confirms that all samples obtained from the field were associated with PepYLCIV infection. Grabagan, Jatirogo, Bancar, and Soko isolates constituted into one subgroup since they were from one district. Isolates from the same region or district can be expected to be in the same subgroup. It was similar to the results from Santosa *et al.* (2024) who reported that two PepYLCIV isolates from the Ngablak Subdistrict, Magelang Regency, belong to the same subgroup.

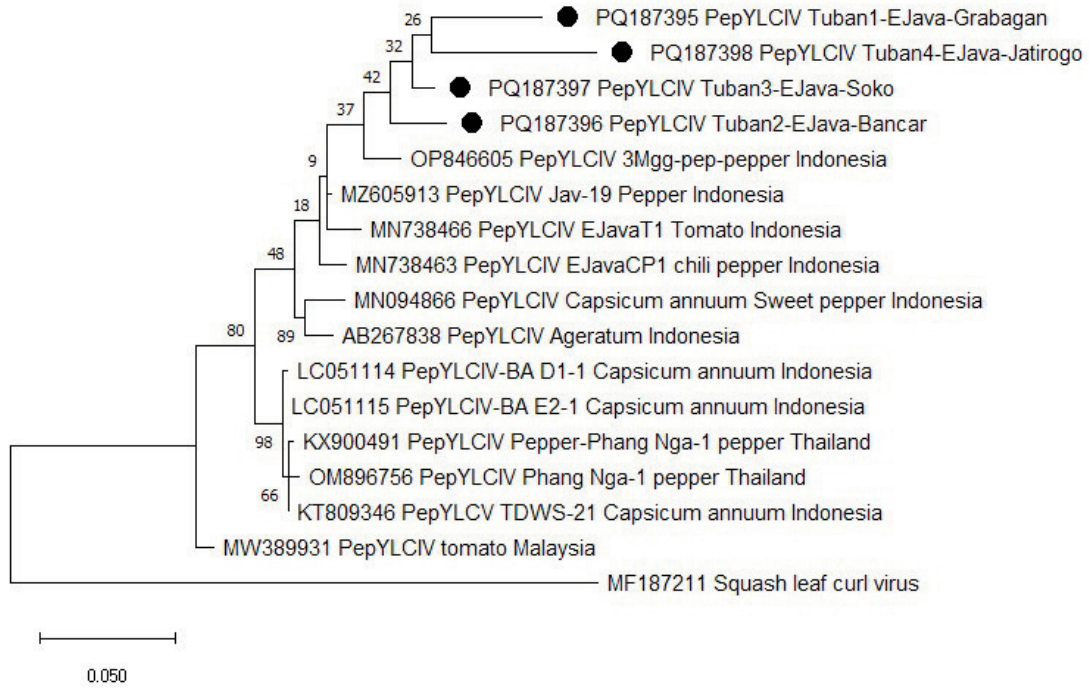

Figure 2. Phylogenetic diagram of PepYLCIV in Soko, Jatirogo, Bancar, and Grabagan compared to other PepYLCIV isolates available in the GenBank.

Table 2. Similarity percentage (homology) of Bancar, Soko, Jatirogo, and Grobogan, Tuban, Indonesia code sample sequences compared to isolates from GenBank

No.	PepYLClV Identity	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	**MZ605913**_Java-Indonesia	**ID**															
2	**OP846605**_ Magelang_ Central Java_Indonesia	97.6	**ID**														
3	**MN738466**_ East Java_ Indonesia	98.5	96.1	**ID**													
4	**MN738463**_ East Java_ Indonesia	98.5	96.1	97.4	**ID**												
5	**MN094866**_ Indonesia	96.7	94.5	96.3	96.3	**ID**											
6	**AB267838**_ Bandung_West Java_ Indonesia	97.2	94.9	96.3	96.5	97.4	**ID**										
7	**LC051115**_Nothern Sumatra_Indonesia	96.3	94.7	95.2	96.1	95.8	96.1	**ID**									
8	**LC051114**_Nothern Sumatra_Indonesia	96.1	94.5	95.0	95.8	95.6	95.8	99.8	**ID**								
9	**OM896756**_ Phanga Nga_Thailand	95.6	94.0	94.5	95.4	95.1	95.4	99.4	99.1	**ID**							
10	**KT809346**_West Sumatra_Indonesia	96.1	94.5	95.0	95.8	95.6	95.8	99.8	99.6	99.6	**ID**						
11	**MW389931**_Malaysia	94.5	93.5	93.3	94.3	94.0	94.2	96.0	95.8	95.4	95.8	**ID**					
12	**KX900491**_ Phanga Nga_Thailand	95.8	94.2	94.7	95.6	95.4	95.6	99.6	99.4	99.4	99.8	95.6	**ID**				
13	**PQ187395**_Grabagan_ Tuban_ East Java	92.6	93.3	91.9	92.8	92.3	92.3	93.7	93.5	93.0	93.5	92.8	93.8	**ID**			
14	**PQ187396**_Bancar_ Tuban_East Java	95.8	96.0	94.7	95.2	94.0	94.2	95.6	95.4	94.9	95.4	94.7	95.1	94.0	**ID**		
15	**PQ187397**_Soko_ Tuban_East Java	96.1	91.0	95.9	95.4	94.7	94.5	94.9	94.7	94.2	94.7	93.7	94.5	94.7	96.3	**ID**	
16	**PQ187398**_Jatirogo_ Tuban_East Java	90.8	91.0	90.1	90.1	89.8	89.6	89.8	89.6	89.1	89.6	88.8	89.3	89.8	92.3	92.5	**ID**

CONCLUSION

Yellow leaf curl disease on chili pepper plants in four subdistricts in Tuban, including Grabagan, Bancar, Soko, and Jatirogo, was caused by PepYLCIV. This was based on the symptoms observation, PCR results, and similarity percentage (homology). All isolates from Tuban (PQ187395 - PQ187398) were closely related and in a same group with the PepYLCIV isolates from Ngablak Subdistrict, Magelang Regency, Central Java, Indonesia (OP846605). Meanwhile, isolates from Tuban formed a separate group to PepYLCV from Malaysia (MW389931).

ACKNOWLEDGMENT

The authors are grateful to *Direktorat Akademik Pendidikan Tinggi Vokasi* (DAPTV), *Direktorat Jenderal Pendidikan Tinggi Vokasi* (DIKSI), and *Kementerian Pendidikan, Kebudayaan, Riset, dan Teknologi* (KEMDIKBUDRISTEK) for their financial support through the scheme "Penelitian Dosen Pemula"

with master contract 104/SPK/D.D4/PPK.01.APTV/III/2024.

LITERATURE CITED

Ayu, D.K., Maharijaya, A., Syukur, M., & Hidayat, S.H. (2021). Resilience Response to Yellow Leaf Curl Disease and Identification of Resistance Gene Analogs (RGA) in Pepper (*Capsicum annuum*). *Biodiversitas*, *22*(11), 4731−4739. https://doi.org/10.13057/biodiv/d221104

Badan Pusat Statistik Provinsi Jawa Timur. (2023). *Produksi Tanaman Sayuran Cabai Besar, Cabai Rawit, Cabai Keriting Menurut Kabupaten/Kota dan Jenis Tanaman di Provinsi Jawa Timur (Kuintal), 2021 dan 2022*. Retrieved from https://jatim.bps.go.id/id/statistics-table/1/MjUzNiMx/-produksi-tanaman-sayuran-cabai-besar-cabai-rawit-cabai-keriting-menurut-kabupaten-kota-dan-jenis-tanaman-di-provinsi-jawa-timur-kuintal-2021-dan-2022.html

Cania, D., Nova, B., Runifah, T., Hidayati, R., Anwar, A., & Jamsari, J. (2021). Molecular Diversity of Pepper yellow leaf curl virus (PepYLCV) Infecting *Capsicum annuum* in West Sumatra. I*OP Conference Series: Earth and Environmental Science*, *741*(1), 012038. https://doi.org/10.1088/1755-1315/741/1/012038

Fadhila, C., Lal, A., Vo, T.T., Ho, P.T., Hidayat, S.H., Lee, J., Kil, E-J., & Lee, S. (2020). The Threat of Seed-transmissible Pepper yellow leaf curl Indonesia virus in Chili Pepper. *Microbial Pathogenesis*, *143*, 104132. https://doi.org/10.1016/j.micpath.2020.104132

Folimonova, S.Y., & Tilsner, J. (2018). Hitchhikers, Highway Tolls and Roadworks: The Interactions of Plant Viruses with the Phloem. *Current Opinion in Plant Biology*, *43*, 82−88. https://doi.org/10.1016/j.pbi.2018.02.001

Gaswanto, R., Syukur, M., Hidayat, S.H., & Gunaeni, N. (2016). Identifikasi Gejala dan Kisaran Inang Enam Isolat *Begomovirus* Cabai di Indonesia [Symptom and Host Range Identification of Six Chilli Begomovirus Isolate in Indonesia]. *Jurnal Hortikultura*, *26*(2), 223−234.

Kandito, A., Hartono, S., Sulandari, S., & Somowiyarjo, S. (2021). A Recombinant DNA-satellite Associated with Pepper yellow leaf curl Indonesia virus in Highland Area. *Indonesian Journal of Biotechnology*, *26*(2), 82−90. https://doi.org/10.22146/IJBIOTECH.64817

Kesumawati, E., Okabe, S., Homma, K., Fujiwara, I., Zakaria, S., Kanzaki, S., & Koeda, S. (2019). Pepper yellow leaf curl Aceh virus: A Novel Bipartite *Begomovirus* Isolated from Chili Pepper, Tomato, and Tobacco Plants in Indonesia. *Archives of Virology*, *164*(9), 2379−2383. https://doi.org/10.1007/s00705-019-04316-8

Nalla, M.K., Schafleitner, R., Pappu, H.R., & Barchenger, D.W. (2023). Current Status, Breeding Strategies and Future Prospects for Managing Chilli Leaf Curl Virus Disease and Associated Begomoviruses in Chilli (*Capsicum* spp.). *Frontiers in Plant Science*, *14*, 1223982. https://doi.org/10.3389/fpls.2023.1223982

Nigam, D. (2021). Genomic Variation and Diversification in *Begomovirus* Genome in Implication to Host and Vector Adaptation. *Plants*, *10*(8), 1706. https://doi.org/10.3390/plants10081706

Revill, P.A., Ha, C.V., Porchun, S.C., Vu, M.T., & Dale, J.L. (2003). The Complete Nucleotide Sequence of Two Distinct Geminiviruses Infecting Cucurbits in Vietnam. *Archives of Virology*, *148*(8), 1523−1541. https://doi.org/10.1007/s00705-003-0109-6

Rubio, L., Galipienso, L., & Ferriol, I. (2020). Detection of Plant Viruses and Disease Management: Relevance of Genetic Diversity and Evolution. *Frontiers in Plant Science*, *11*, 01092. https://doi.org/10.3389/fpls.2020.01092

Santosa, A.I., Irbati, A.H., Pratiwi, G.N.C., Surwadinata, A., Laeshita, P., Dharma, K.S., Jaya, R.S., Andriyani, A.L., A'yun, C.Q., & Fatika, C.N.S. (2024). Updates on Hosts and Distribution of Pepper yellow leaf curl Indonesia virus and Squash leaf curl China virus in Central Java Province, Indonesia. *Jurnal Ilmiah Pertanian*, *21*(1), 11−20. https://doi.org/10.31849/jip.v21i1.18856

Sayekti, T.W.D.A., Syukur, M., Hidayat, S.H., & Maharijaya, A. (2021). Morphological Response and Genetic Variability of Four Species of Chili Pepper (*Capsicum* spp.) under Infection of Pepper yellow leaf curl virus. *Biodiversitas*, *22*(11), 4758−4765. https://doi.org/10.13057/biodiv/d221107

Selangga, D.G.W., & Listihani. (2021). Molecular Identification of Pepper yellow leaf curl Indonesia virus on Chili Pepper in Nusa Penida Island. *Journal of Tropical Plant Pests and Diseases*, *21*(2), 97−102. https://doi.org/10.23960/jhptt.22197-102

Sulandari, S. (2006). Penyakit Daun Keriting Kuning Cabai di Indonesia [Pepper Yellow Leaf Curl Disease in Indonesia]. *Jurnal Perlindungan Tanaman Indonesia*, *12*(1), 1−12. Retrieved from https://jurnal.ugm.ac.id/jpti/article/view/11941

Temaja, I.G.R.M., Selangga, D.G.W., Phabiola, T.A., Khalimi, K., & Listihani. (2022). Relationship between Viruliferous *Bemisia tabaci* Population and Disease Incidence of Pepper yellow leaf curl Indonesia virus in Chili Pepper. *Biodiversitas*, *23*(10), 5360−5366. https://doi.org/10.13057/biodiv/d231046

Temaja, I.G.R.M., Selangga, D.G.W., Sudiarta, I.P., & Listihani. (2024). Antiviral Activity of *Eucheuma cottonii* to Control Pepper yellow leaf curl virus. *Biodiversitas*, *25*(4), 1615−1622. https://doi.org/10.13057/biodiv/d250430

Wahyono, A., Murti, R.H., Hartono, S., Nuringtyas, T.R., Wijonarko, A., Mulyantoro, M., Firmansyah, D., Afifuddin, A., & Purnama, I.C.G. (2023). Current Status and Complexity of Three Begomovirus Species in Pepper Plants in Lowlands and Highlands in Java Island, Indonesia. *Viruses*, *15*(6), 1278. https://doi.org/10.3390/v15061278

Widodo, C.J., Taufik, M., Khaeruni, A., & Mallarangeng, R. (2023). Determination of *Begomovirus* on Chili Plants (*Capsicum* sp.) in Buton and Muna Islands, Southeast Sulawesi, Indonesia. *Biodiversitas*, *24*(2), 741−751. https://doi.org/10.13057/biodiv/d240209

Windarningsih, M. (2019). Identification of Virus Causing the Yellow Leaf Curl Diseases on Chili Pepper in Lombok Island by PCR-RFLP Technique. *AIP Conference Proceedings*, *2199*, 040010. https://doi.org/10.1063/1.5141297

Yolanda, K., Witjaksono., & Putra, N.S. (2024). Population of *Bemisia tabaci* and Incidence of Yellow Disease in Chili Intercropped with Corn. *Jurnal Perlindungan Tanaman Indonesia*, *28*(1), 68−76. https://doi.org/10.22146/jpti.88503