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Detection and Sequence Analysis of Pepper Yellow Leaf Curl Virus Isolates That Infected Chili (*Capsicum annuum* L.) in Bengkulu, Indonesia

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ABSTRACT: Pepper yellow leaf curl Aceh virus (PepYLCAV) and pepper yellow leaf curl Indonesia virus (PepYLCIV) are begomoviruses that dominate chili cultivation in Indonesia. Characterization of these two begomoviruses is essential as basic information for the development of virus control technologies. The symptoms on chili plants indicate that PepYLCIV is more severe than PepYLCAV, with severe mosaic, curling, and yellowing, whereas PepYLCAV causes only mild mosaic and yellowing. Sequencing results also show that the nucleotides and amino acids between PepYLCAV and PepYLCIV are significantly different. This suggests that the pathogenicity of the two viruses on chili plants differs. Protein structure predictions using AlphaFold3 also show significant differences. Based on available data, control of PepYLCAV and PepYLCIV can be achieved using different approaches, as these viruses have distinct sequences that may affect their pathogenicity.

Keywords: Capsicum annuum L., pepper yellow leaf curl virus, sequence

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INTRODUCTION

Chili (*Capsicum annuum* L.) is an essential crop in Indonesia, with high economic and nutritional value (Orellana-Escobedo and Lopez-Maltez, 2022). The high consumption and unstable production of chili peppers lead to significant fluctuations in market prices (Nauli, 2016). Chili pepper productivity is greatly influenced by abiotic and biotic factors (Chhapekar et al., 2018). Biotic factors such as pathogen attacks greatly affect the growth and yield of chili peppers in the field (Syamsidi et al., 1997). Hidayat et al. (1999) virus attacks can cause yield losses of up to 100%.

One of the viruses important to chili plants is begomovirus. Begomoviruses are DNA viruses that cause significant economic losses worldwide (García-Arenal and Zerbini, 2019). Begomovirus has a wide host range, including Solanaceae plants (Nigam, 2021). Begomovirus causes annual economic losses from 2-140 billion US dollars in tomatoes, cotton, and beans (Moffat, 1999; Faria and Maxwell, 1999; Varma and Malathi et al., 2003). The genomes of bipartite begomoviruses consist of two components, DNA-A and DNA-B, each of 2.5–2.6 kb. The DNA-A component of the bipartite begomoviruses can replicate autonomously and produce virions. The DNA-B component is required for systemic infection.

The organization of ORFs in the genomes of monopartite begomoviruses is similar to the bipartite viral DNA-A component (Hanley-

Bowdoin et al., 1999). Begomovirus transmission is an essential factor in virus spread in the field. Most begomoviruses are transmitted by whitefly (Fiallo-Olive et al., 2021). Whether begomoviruses replicate in whiteflies (circulative, propagative) or do not replicate in whiteflies (circulative, non-propagative) remains debated (Becker et al., 2015; Pakkianathan et al., 2015; Sanchez-Campos et al., 2016; Wang et al., 2016). Besides transmission via whiteflies, begomoviruses can also be mechanically transmitted (Lee et al., 2020).

Begomovirus control has been implemented through various methods, including the identification of resistance genes (Ji et al., 2009). Koda et al. (2021) found that pyramiding the *pepy-1* and *pepy-2* genes can suppress the symptoms and accumulation of PepYLCIV/PepYLCAV in chili plants. *pepy-1* is identified as an *RNA-dependent RNA polymerase* (*RDR*). Identification of resistance genes is carried out by analyzing host-pathogen interactions (Ko and Smith, 2020). Molecular characterization and pathogen identification are essential for developing control methods for begomoviruses in chili plants. The Pepper yellow leaf curl Aceh virus (PepYLCAV) and the Pepper yellow leaf curl Indonesia virus (PepYLCIV) are prevalent in chili cultivation in Indonesia. Aulia et al. (2022) first reported PepYCIV in chili peppers in Bengkulu. To control PepYLCAV and PepYLCIV, they must be characterized.

MATERIALS AND METHODS

Samples Collection

Samples were collected from chili plants grown in the plant protection laboratory experimental field at Bengkulu University. Chili plants showing systemic yellowing and leaf curling were collected and placed in 1.5mL Eppendorf tubes. Photographs of the sampled plants were taken and immediately stored in a freezer at -80°C. Samples were removed when they were ready for DNA extraction.

DNA Extraction

DNA extraction was performed on leaf samples using the CTAB method, as described by Sutrawati et al. (2021). DNA quality was checked using a nanodrop. The DNA was stored in a freezer at -20°C.

Polymerase Chain Reaction (PCR)

PCR detection was conducted using genomic DNA with a begomovirus degenerate primer pair (F: GCATCTGCAGGCCCACATYGTCTTYCCNGT, R: GATTTCTGCAGTTDATRTTYTCRTCCATCCA, size: 1,3 kb). PCR was performed in a 50 µl reaction containing 0.25 U ProTaq polymerase (PROTECH, Taipei, Taiwan) and 0.05 mM dNTPs. Healthy DNA extracted from healthy chili pepper was used as a negative control.

Sanger Sequencing

The PCR products were sent to the Biotechnology Center, National Chung Hsing University, Taiwan, for Sanger sequencing. The sequence results were analyzed with SeqBuilder and blasted against NCBI.

Protein Structure Prediction

The DNA sequences were translated into protein by SeqBuilder. Protein sequences were input into AlphaFold3 (AlphaFold Server). The results were analyzed using PyMOL.

RESULTS AND DISCUSSION

TYLCKaV detection by PCR

Chili plants in the experimental field exhibited mosaic, yellowing, and curling symptoms (Figure 1). The severity of the symptoms varied, ranging from mild (Figure 1a) to severe (Figure 1b). This indicates a high degree of variation in virus attacks in the field. The symptoms of viral diseases in plants depend on the species of virus that infects them, the level of virus accumulation in plant tissues, and viral interactions (Gao and Lozano-Duran, 2025). Different virus species exhibit distinct genetic characteristics, resulting in a range of symptoms from mosaic spots and chlorosis to stunted growth. High virus accumulation can exacerbate tissue damage, making symptoms more pronounced and severe (Kundu et al., 2024). Additionally, when plants are infected with multiple virus types, viral interactions can cause more severe symptoms or symptoms that differ from those of a single infection. Thus, the complexity of virus species, accumulation levels, and interactions significantly determines symptom manifestation in virus-infected plants (DaPalma et al., 2010).

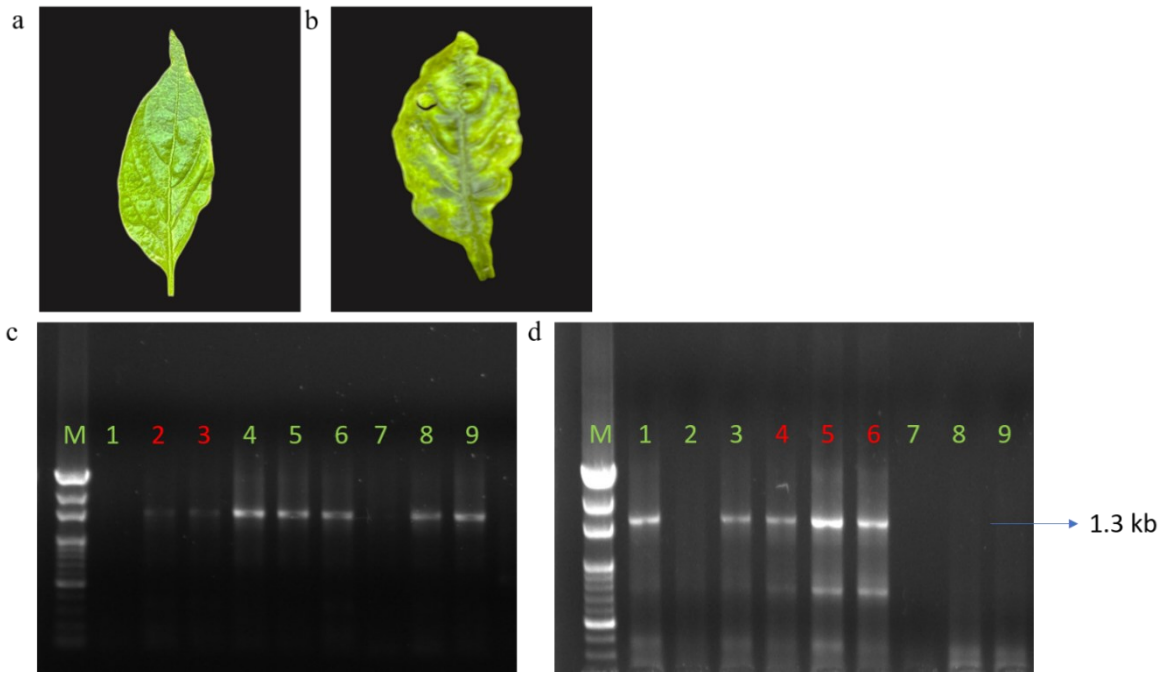

Figure 1. Symptoms observation and virus detection by PCR using begomovirus degenerate primer. M: Marker 100 bp, red number indicated the samples were sent for Sanger sequencing. a and b: symptoms observed in chili plants, c and d: PCR results of begomovirus detection.

Figures 1c and 1d showed that the symptomatic leaves were infected with begomovirus. The PCR results showed DNA bands measuring 1.2 to 1.3 kb. These PCR reactions used a degenerate primer; therefore, the PCR products were sent for Sanger sequencing.

Sequencing results and analysis

Sequencing results showed that samples 2 and 3 in Figure 1c were infected with pepper yellow leaf curl Aceh virus. The identity reached 97% (Table 1). Kesumawati et al. (2017) first reported Pepper yellow leaf curl Aceh virus (PepYLCAV) in Aceh Province, Indonesia. The study identified a new begomovirus isolate in chili (*Capsicum annuum*), tomato (*Solanum lycopersicum*), and tobacco (*Nicotiana tabacum*) plants exhibiting leaf yellowing and curling symptoms. The DNA genome sequence of this isolate is highly like that of the pepper yellow leaf curl Indonesia virus (PepYLCIV).

Samples 4, 5, 6 in Figure 1d were infected by pepper yellow leaf curl Indonesia virus (PepYLCIV) based on BLAST results (Table 1). PepYLCIV was also reported by Selangga et al. (2021) in infected chili pepper in Bali. In Bengkulu, PepYLCIV was first reported by Sutrawati et al. (2022) in chili plants. PepYLCAV and PepYLCIV are the most critical begomoviruses in Chili (Taufik et al., 2023).

Table 1. BLAST results of begomovirus PCR product sequencing

Samples	BLAST Results (NCBI-Top Result)	Percent Identity	Accesion
Samples 2,3	**Pepper yellow leaf curl Aceh virus PepYLCAV-BATa-9 genomic DNA, segment: DNA-A, complete sequence**	97%	LC465989
Samples 4, 5, 6	**Pepper yellow leaf curl Indonesia virus C1, C4, V2, V1 genes for replication-associated protein, putative C4 protein, putative V2 protein, coat protein, partial and complete cds**	92%	AB246170

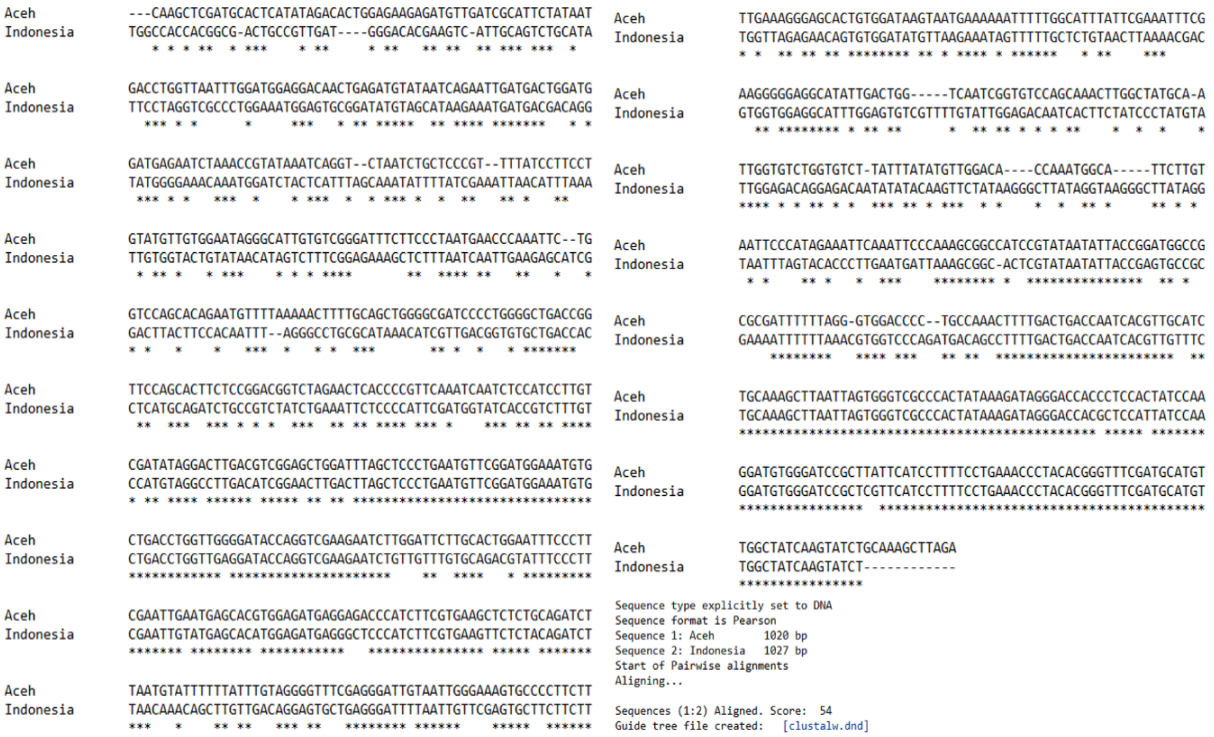

Figure 2. Nucleotide comparison of AC1-AV1 of pepper yellow leaf curl Aceh virus (PepYLCAV) and pepper yellow leaf curl Indonesia virus (PepYLCIV).

The alignment sequence AC1-AV1 PepYLCAV and PepYLCIV infecting chili peppers in Bengkulu shows several nucleotide differences. CLUSTALW shows that the similarity between AC1 and AV1 is only 54%, with a genome coverage of 1.2 kb. This indicates that the Bengkulu isolates PepYLCIV and PepYLCAV differ significantly from other isolates. According to Koeda et al. (2019), PepYLCIV and PepYLCAV should exhibit high sequence similarity, suggesting that the PepYLCIV and PepYLCAV isolates from Bengkulu may differ in pathogenicity and virulence compared to other isolates.

Genetic variation affects a virus's ability to infect certain plant types, produce different symptoms, and determine the severity of the disease in the host. Isolates of the Pepper yellow leaf curl Indonesia virus (PYLCIV) from Java exhibit high genomic similarity to isolates from West Sumatra (Taufik et al., 2023). However, these isolates differ in their ability to infect plants besides chili peppers, including tomatoes and *Ageratum conyzoides*. The PYLCIV isolate from Padang can infect tomatoes and peppers, whereas isolates from other regions have a more limited host range, infecting only peppers (Trisno et al., 2010). These differences are caused by variations in the coat protein and replication regulator genes, which influence specific virus-host interactions.

TYLCKaV protein structure prediction

The differences in nucleotides between PepYLCAV and PepYLCIV caused a change in amino acids due to a single nucleotide polymorphism (SNP) when translated into protein (Figure 3). SNPs in the coding region of a gene can alter a protein's amino acid sequence, a change known as a nonsynonymous SNP (Robert and Pelletier, 2018). These changes can alter protein function by disrupting stability, enzymatic activity, or protein-protein interactions (Ramirez-Bello and Jimenez-Morales, 2017). Amino acid changes due to SNPs can cause proteins to lose their normal function or acquire new functions that may lead to disease (Shastry et al., 2009). Therefore, SNPs play an essential role in differences in the individual biological response, including disease susceptibility.

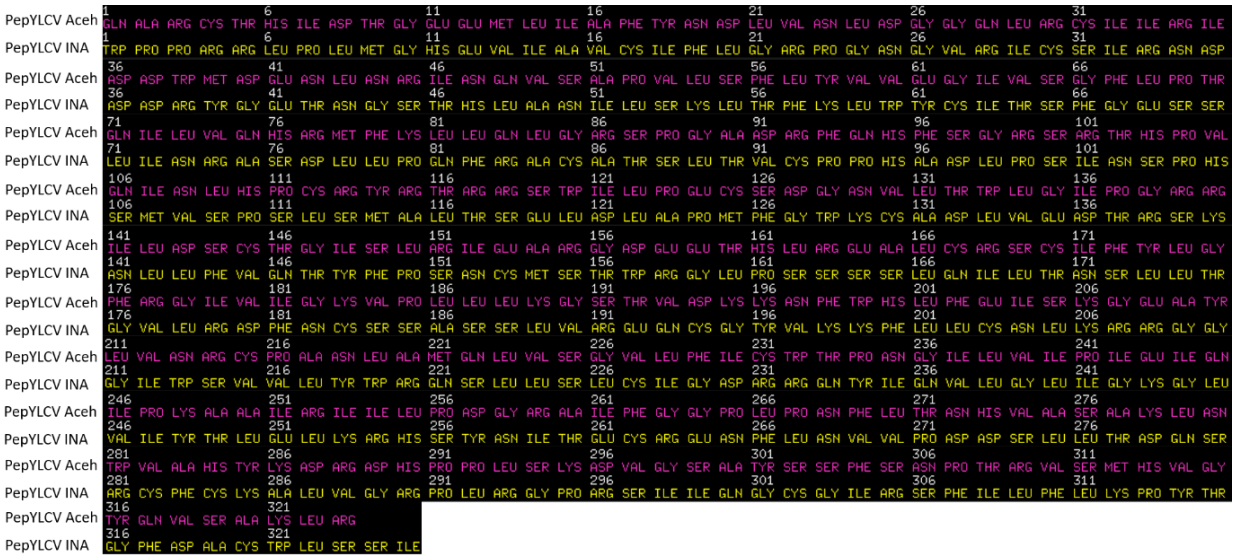

Figure 3. Amino acids comparison between PepYLCAV (pink) and PepYLCIV (yellow).

PepYLCIV has more extended amino acid sequences than PepYLCAV. To determine the more specific effects of these differences, we predicted the protein structure using AlphaFold3 (Figure 4).

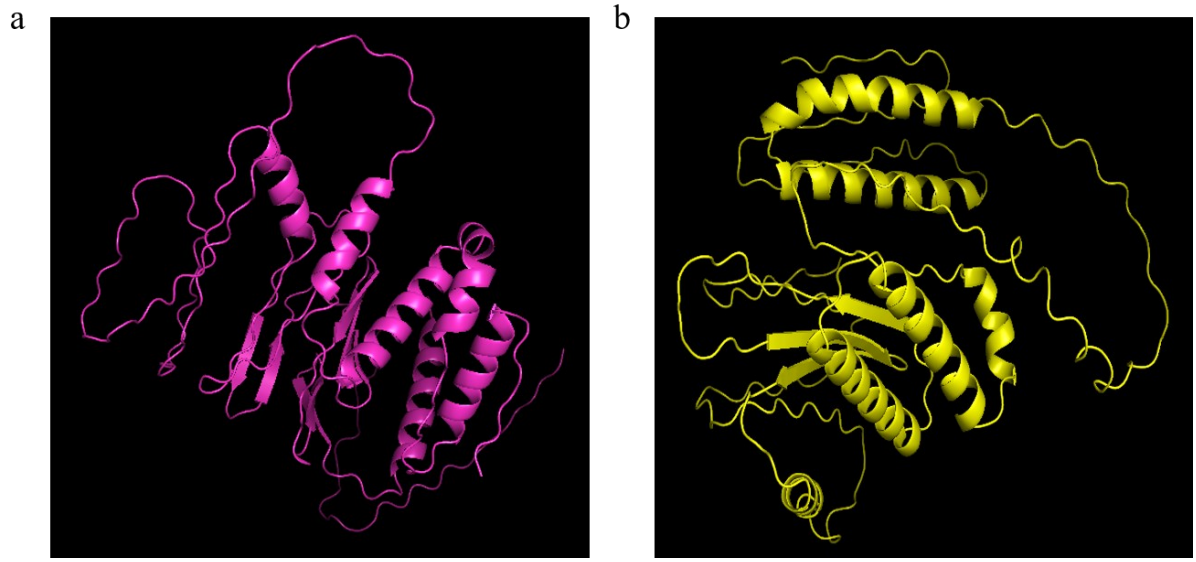

Figure 4. protein structure prediction by AlphaFold3. a. Protein structure of PepYLCAV, b. Protein structure of PepYLCIV.

As shown in Figure 4, the PepYLCAV and PepYLCIV protein structures are slightly different. This difference is expected to affect their interaction with the host. A virus protein's structure plays a central role in determining how the virus interacts with other viruses (in cases of co-infection or synergism), and with the plant host cells it targets (Smith, 2018). Proteins in the capsid (the protective layer) and non-structural proteins synthesized by the virus function as molecular keys. Interaction with host cells begins when viral surface proteins recognize and bind to specific receptors on the plant cell membrane. Slight differences in protein folding or amino acid sequence can determine host specificity, or the type of plant that can be infected (Jones & Brown, 2020). Furthermore, movement proteins facilitate the transfer of viruses from one cell to another

through plasmodesmata; their alteration can limit or expand systemic spread (White et al., 2019). In coinfections, viral proteins can interact positively or negatively.

CONCLUSION

PepYLCAV and PepYLCIV have been detected in chili plants in Bengkulu, as confirmed by PCR and sequencing. These two viruses have different symptoms and spread in Bengkulu's chili plantations. Differences in their sequences also lead to amino acid SNPs, which affect the predicted protein structure using AlphaFold3.

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