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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 replicate bipartite begomoviruses can 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 various methods, including through 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 RNAdependent RNA polymerase (RDR). Identification of resistance genes is carried out by analyzing hostpathogen interactions (Ko and Smith, 2020). Molecular characterization and pathogen identification are essential for developing control methods for begomoviruses in chili plants. The Pepper vellow 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 mosaic, yellowing, and curling exhibited 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, significantly interactions determines symptom manifestation in virus-infected plants (DaPalma et al., 2010).

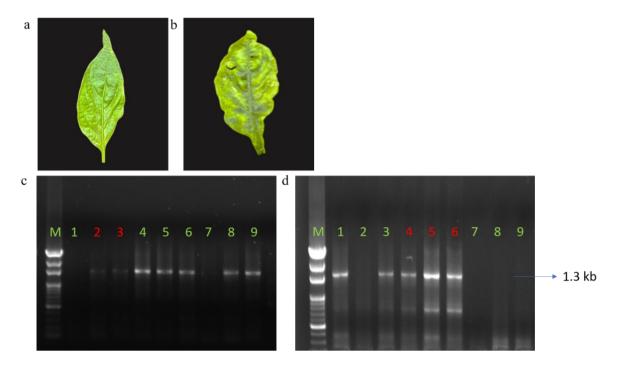


Figure 1. Symptoms observation and virus detection by PCR using begomovirus degenerate primer. M: Marker 100 bp, red number indicated the samples were sent for Sanger sequencing. a and b: symptoms observed in chili plants, c and d: PCR results of begomovirus detection.

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 replicationassociated protein, putative C4 protein, putative V2 protein, coat protein, partial and complete cds	92%	AB246170

Aceh Indonesia	CAAGCTCGATGCACTCATATAGACACTGGAGAAGAGATGTTGATCGCATTCTATAAT TGGCCACCACGGCG-ACTGCCGTTGATGGGACACGAAGTC-ATTGCAGTCTGCATA * * * * * * * * * * * * * * * * * * *	Aceh Indonesia	TTGAAAGGGAGCACTGTGGATAAGTAATGAAAAAATTTTTGGCATTTATTCGAAATTTCG TGGTTAGAGAACAGTGTGGATATGTTAAGAAATAGTTTTTGCTCTGTAACTTAAACGAC
Aceh Indonesia	GACCTGGTTAATTTGGATGGAGGACAACTGAGATGTATAATCAGAATTGATGACTGGATG TTCCTAGGTCGCCCTGGAAATGGAGTGCGGATATGTAGCATAAGAAATGATGACGACAGG	Aceh Indonesia	AAGGGGGAGGCATATTGACTGGTCAATCGGTGTCCAGCAAACTTGGCTATGCA-A GTGGTGGAGGCATTTGGAGTGTCGTTTTGTATTGGAGACAATCACTTCTATCCCTATGTA
Aceh Indonesia	GATGAGAATCTAAACCGTATAAATCAGGTCTAATCTGCTCCCGTTTTATCCTTCCT TATGGGGAAACAAATGGATCTACTCATTTAGCAAATATTTTATCGAAATTAACATTTAAA *** * * *** * * *** * * *** * * *** * *	Aceh Indonesia	TTGGTGTCTGGTGTCT-TATTTATATGTTGGACACCAAATGGCATTCTTGT TTGGAGACAGGACAATATATACAAGTTCTATAAGGGCTTATAGGTAAGGGCTTATAGG
Aceh Indonesia	GTATGTTGTGGAATAGGGCATTGTGTCGGGATTTCTTCCCTAATGAACCCAAATTCTG TTGTGGTACTGTATAACATAGTCTTTCGGAGAAAGCTCTTTAATCAATTGAAGAGCATCG * ** * * * *** * * * * * * * * * * * *	Aceh Indonesia	AATTCCCATAGAAATTCAAATTCCCAAAGCGGCCATCCGTATAATATTACCGGATGGCCG TAATTTAGTACACCCTTGAATGATTAAAGCGGC-ACTCGTATAATATTACCGAGTGCCGC
Aceh Indonesia	GTCCAGCACAGATGTTTTAAAAACTTTTGCAGCTGGGGCGATCCCCTGGGGCTGACCGG GACTTACTTCCACAATTTAGGGCCTGCGCATAAACATCGTTGACGGTGTGCTGACCAC * * * * *** * * * ****	Aceh Indonesia	CGCGATTTTTTAGG-GTGGACCCCTGCCAAACTTTTGACTGACCAATCACGTTGCATC GAAAATTTTTTAAACGTGGTCCCAGATGACAGCCTTTTGACTGAC
Aceh Indonesia	TTCCAGCACTTCTCCGGACGGTCTAGAACTCACCCCGTTCAAATCAATC	Aceh Indonesia	TGCAAAGCTTAATTAGTGGGTCGCCCACTATAAAGATAGGGACCACCCTCCACTATCCAA TGCAAAGCTTAATTAGTGGGTCGCCCACTATAAAGATAGGGACCACGCTCCATTATCCAA
Aceh Indonesia	CGATATAGGACTTGACGTCGGAGCTGGATTTAGCTCCCTGAATGTTCGGATGGAAATGTG CCATGTAGGCCTTGACATCGGAACTTGACTTAGCTCCCTGAATGTTCGGATGGAAATGTG * * * * * * * * * * * * * * * * * * *	Aceh Indonesia	GGATGTGGGATCCGCTTATTCATCCTTTTCCTGAAACCCTACACGGGTTTCGATGCATGT GGATGTGGGATCCGCTCGTTCATCCTTTTCCTGAAACCCTACACGGGTTTCGATGCATGT
Aceh Indonesia	CTGACCTGGTTGGGGATACCAGGTCGAAGAATCTTGGATTCTTGCACTGGAATTTCCCTT CTGACCTGGTTGAGGATACCAGGTCGAAGAATCTGTTGTTTGT	Aceh Indonesia	TGGCTATCAAGTATCTGCAAAGCTTAGA TGGCTATCAAGTATCT
Aceh Indonesia	CGAATTGAATGAGCACGTGGAGATGAGGAGACCCATCTTCGTGAAGCTCTCTGCAGATCT CGAATTGTATGAGCACATGGAGATGAGGGCTCCCATCTTCGTGAAGTTCTCTACAGATCT	Sequence format 1: Sequence 1: Aceh Sequence 2: Indon Start of Pairwise	1020 bp esia 1027 bp
Aceh Indonesia	TAATGTATTTTTTATTGTAGGGGTTTCGAGGGATTGTAATTGGGAAAGTGCCCCTTCTT TAACAAACAGCTTGTTGACAGGAGTGCTGAGGGATTTTAATTGTTCGAGTGCTTCTTCTT ***		ligned. Score: 54 reated: [clustalw.dnd]

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 similarity between AC1 and AV1 is only 54%, with a genome coverage of 1.2 kb. This indicates that the Bengkulu isolates PepYLCIV 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 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 function disrupting by stability, enzymatic activity, or protein-protein interactions (Ramirez-Bello and Jimenez-Morales, 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.

DVI CV A			6					11					16					21					26					31				
PepYLCV Aceh	.N ALA ARG I	CYS 1	THR H	IS IL	E ASP	THR	GLY	GLU	GLU I	MET	LEU	ILE	ALA	PHE	TYR			LEU			LEU		GLY	GLY	GLN	LEU		CYS	ILE	ILE		ILE
PepYLCV INA	P PRO PRO	ARG A	ARG L	EU PR	O LEU	MET	GLY	HĪS	GLU 1	VAL	ILE	ALA	VAL	CYS	ILE	PHE	LEU	ĞĹY	ARG	PRO	GLY	ASN	ĞĽY	VAL	ARG	ILE	CYS	SER	ILE	ARG	ASN	ASP
PepYLCV Aceh	6		,	1				46					51					56					61					66				
Pepylcv Acen	SP ASP TRP	MET	ASP (	iLU A!	SN LEI	J ASN		ILE 46		GLN		SER	ALA	PRO		LEU	SER	PHE	LEU	TYR			GLU 61	GLY	ILE		SER	GLY	PHE	LEU	PRO '	THR
PepYLCV INA	6 SP ASP ARG	TYR	GLY (	LU T	IR ASI	4 GLY	SER	THR	HIS	LEU	ALA	ASN	ĬĹE	LEU	SER	LYS	LEU	THR	PHE	LYS	LEU	TRP	TŸR	CYS	ILE	THR	SER	PHE	GLY	GLU	SER :	SER
PepYLCV Aceh	1			6				81					86					91					96					101				
7	LN ILE LEU 1		GLN F	122 HI	KG ME	PHE	LYS	81	LEU	GLN	LEU	GLY	86	SER	PRU	GLY	HLH	91		PHE	GLN	HIS	96	SER	GLY		SER	101		H12	PRU	VHL
	EU ILE ASN							GLN																				ILE				
PepYLCV Aceh	06		UTC I	111				116					121					126		0.0			131					136				ODC.
10	06			111				116					121					126					131					136				
	ER MET VAL	SER			EU SEI	R MET	ALA		THR	SER	GLU	LEU		LEU	ALA	PRO	MET		GLY	TRP	LYS	CYS		ASP	LEU	VAL			THR	ARG	SER	LYS
PepYLCV Aceh	41		cve i	.46	V TI I			151	TIE		01.0		156					161				01.0	166					171			LEIL	CI V
14	41		1	46				151					156					161					166					171				
	SN LEU LEU 76	PHE	VAL (	LN TI	IR TY	R PHE	PRO	SER	ASN	CYS	MET	SER	THR	TRP	ARG	GLY	LEU	PRO	SER	SER	SER	SER	LEU 201	GLN	ILE	LEU	THR	206	SER	LEU	LEU '	THR
PepYLCV Aceh	HE ARG GLY	ILE		LE GI		S VAL	PRO	186 LEU	LEU	LEU	LYS	GLY	191 SER			ASP	LYS	196 LYS		PHE	TRP	HIS	LEU	PHE	GLU	ILE	SER	LYS	GLY	GLU	ALA	TYR
1	76		1	81	SN CY			186					191					196				-	201					206				
PepYLCV INA	LY VAL LEU	HRG		16	SN CY	5 SER	SER	221	SER	SER	LEU	VHL	226	GLU	GLN	CYS	GLY	231	VHL	LYS	LYS	PHE	236	LEU	CYS	HSN	LEU	241	HRG	HRG	GLY	GLY
PepYLCV Aceh	U VAL ASN	ARG I	CYS F			l LEU	ALA	MET	GLN	LEU			GLY		LEU	PHE	ILE	CYS			PRO		GLY	ILE	LEU		ILE	PRO	ILE	GLU	ILE	GLN
21	L1 _Y ILE TRP	cen s	201 2	16	II TO		ODC	221	CED			CED	226	cvc	T1 5	CI V	ocn.	231	onc.	CLN	TVD	T1 E	236	WAL		CI V		241	CI V	1.20	CLV	
PepYLCV INA	AG ILE IRF	OEK 1		HL LE	.0 111	IRF	HKG	256	SER	LEU	LEU	SER	261	LTS	ILE	GLT	нэг	266	HKG	GEN	HK	ILE	271	VHL	LEU	GLT		276	GLT	LIS	GLT	LEU
PepYLCV Aceh			ALA I	LE A				PRO					ILE					LEU									ALA	SER				
PepYLCV INA V	46 AL ILE TYR	THP	LEU (	251	H 1 Y	a opc	нте	256	TYP	ASN	TIF	THP	261	CYS	APC	GLII	ASN	266	LEII	OSN	VOI	Val	271	asp	asp	SEP	LEII	276	THP	OSP	CLN	SEP
. 28	81		- 2	86				291					296					301					306					311				
PepYLCV Aceh	RP VAL ALA		TYR L	YS AS	SP AR	a ASP		PRO	PRO	LEU			ASP		GLY		ALA	TYR			PHE		ASN		THR			SER	MET			GLY
PepYLCV INA	B1 RG CYS PHE	CYS	LYS A	LA LE	U VAL	GLY	ARG	PRO	LEU	ARG	GLY	PRO	ARG	SER	ILE	ILE	GLN	GLY	CYS	GLY	ILE	ARG	SER	PHE	ILE	LEU	PHE	LEU	LYS	PRO	TYR	THR
PepYLCV Aceh	16			21																												
76 TECV ACEII	TR GEN VAL	SER	HEH L	75 LE 21	U ARI																											
PepYLCV INA	Y PHE ASP	ALA			U SEF	SER	ILE																									

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).

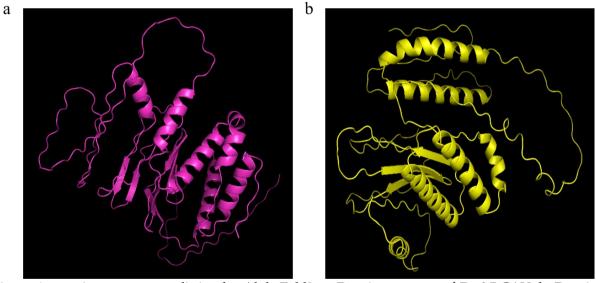


Figure 4. protein structure prediction by AlphaFold3. a. Protein structure of PepYLCAV, b. Protein structure of PepYLCIV.

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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