Molecular diversity of Pepper Yellow Leaf Curl Virus (PepYLCV) Infecting Capsicum annuum in West Sumatra

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Abstract. In recent years, Pepper Yellow Leaf Curl Disease (PepYLCD) is one of the most common diseases affecting chili cultivation in Indonesia, including West Sumatra. In 2019, Pepper Yellow Leaf Curl Virus (PepYLCV) damaged chili plants and have lost yields of up to 100%. The controlling of this PepYLCV that has been carried out so far is less effective because the virus often mutates. In 2019, PepYLCV damaged the chili crop and resulted in 100% yield loss. The controls that have been done so far are less effective, especially because the virus mutates frequently. Genome editing is one of the solutions to control the PepYLCV attacks. For that purpose, information of the genome sequence of the PepYLCV is necessary. This study was aimed to obtain the size of the PepYLCV-APWS genome and the differences in its genomic sequence characteristics with other PepYLCV isolates in West Sumatra. The results showed that PepYLCV has a genome size of 2743 bp. This PepYLCV-APWS isolate had molecular diversity with two other isolates from West Sumatra, PepYLCV TDWS and PepYLCV PSSWS respectively 91% and 92%.

Keywords: Chili, characterization, genome editing, PepYLCV

1. Introduction
Chili is a plant often cultivated and consumed by people in the world. But during cultivation, chili is susceptible to attack by Pepper Yellow Leaf Curl Virus (PepYLCV). PepYLCV has six protein-coding genes are namely C1 encoding replication-initiation protein (Rep), C2 encoding transcriptional activator protein, C3 encoding replication enhancer protein, and C4 encoding protein required for host range determination, symptom severity, and movement of virus were V1 and V2 encoding proteins required for virus movement, host range and pathogenicity [1, 2].

The classical control like the use of superior varieties, crop rotation, planting of hedgerows, use of natural enemies, and use of natural and synthetic insecticides to control this virus. According to previous research, this strategy was also ineffective, mainly because the virus mutates so rapidly that antivirals were not available until now [3]. This mutation is caused by the presence of a vector insect from PepYLCV which can carry more than one virus. This resulted in the emergence of a variety of symptoms in the field and also molecular diversity of PepYLCV.

Previous research has identified two isolates from Tanah Datar and Pesisir Selatan. These two areas are known to be areas with different altitudes, but PepYLCV is still found in both areas [4, 5]. Apart from these two areas, Alahan Panjang is also known to have different topographical conditions from...
the two areas. This is important considering that Alahan Panjang is also a contributor to chili producing areas. Information of molecular diversity can get from compared one isolate with other isolates. Based on this background, this study aimed to isolate and characterized of molecular diversity of PepYLCV infecting Capsicum annum in West Sumatera.

2. Materials and Method

2.1. Plant material and DNA Isolation
Chili plants are suspected of being attacked by PepYLCV scale 3 with the characteristics of yellowing, curving, upward curving leaves, and the plants are still growing [6]. The plants were taken from the Alahan Panjang area in Simpang Tanjung Nan IV, Danau Kembar, Solok Regency. Then the virus DNA was isolated using the ThermoFisher Scientific GeneJET Plant Genomic DNA Purification Mini Kit. Furthermore, the isolated DNA was visualized by agarose electrophoresis 1% (m/v) buffer 0.5x TBE.

2.2. Amplification and Sequencing
To get the complete genome of the isolate Alahan Panjang, PepYLCV detection was done by amplifying DNA with TD21-455F/R primer [4]. Each PCR reaction consisted of the following cocktail: 25 μL KOD One mix Blue, 1 μL F primer, 1 μL R primer, 1 μL DNA template, 22 μL nuclease-free water. Then the PCR results were continued for sequencing analysis.

2.3. Phylogenetic analysis of PepYLCV
The nucleotide sequences obtained were analyzed using Bioedit software and analyzed the level of similarity with other sequences using BLASTn. Then selected ten sequences from the database having sequence identity values of 85-99% and created a phylogenetic tree. Phylogenetic analysis was performed using MEGA X software. The tree consensus bootstrap was inferred from 1000 replications.

3. Result and Discussion

3.1. DNA isolation of PepYLCV-APWS
Chili plants suspected to be attacked by PepYLCV were taken from Alahan Panjang Simpang Tanjung Nan IV, Danau Kembar, Kab. Solok, West Sumatra. According to Lapidot et al., (2001), plants with these symptoms are included on scale 3 [6]. On this scale, plant cells are still differentiated so that viruses can still develop in plant tissue (Figure 1a).

![Figure 1. a. Morphological of affected chili plants. b. The results of DNA isolation.](image)

Chili plants suspected of being attacked by PepYLCV were first verified by isolating genomic DNA. The results of the chili genomic DNA isolation can be seen in Figure 1b. For the quantity of the sample DNA, the DNA concentration was measured using Biodrop. The concentration of DNA
sample 1 was obtained 382.9 ng / µL with a DNA purity level of 1.8 (A280 / 260). DNA is to be pure if it has an absorbance value between 1.8-2.0 [7]. DNA purity whose value is lower than 1.8 indicates that the DNA sample is contaminated by protein, whereas if the value is higher than 2.0 it means that the DNA sample is contaminated by RNA. DNA samples can be continued for the amplification process.

3.2. DNA isolation of PepYLCV-APWS

The isolated DNA was then continued for amplification using the PepYLCV primer. At this amplification stage, the resulting product is a single band, thick and very bright compared to the marker (Figure 2). This indicates that the amplification carried out resulted in intact DNA. According to [8], intact DNA is characterized by the absence of a DNA smear during electrophoresis. This results indicate that DNA sample 1 contains the West Sumatra strain PepYLCV genome. This shows that the isolation and amplification of PepYLCV-APWS were successful.

![Figure 2. Amplification of PepYLCV-APWS](image)

3.3. Sequencing Analysis

The amplification result then carried out sequencing analysis using the sanger method. The sequencing result was processed using Bioedit software. Based on the sequencing results, the nucleotide length of PepYLCV-APWS was obtained at 2743 bp.

![Figure 3. Elektrophoregram snapshot PepYLCV-APWS](image)

Figure 3 shows that the peak is of fairly good quality. This is influenced by the quality of the sample used is quite good and affects the readings on the sequencer machine. The characteristics of a good electropherogram include: (a). The spacing between the peaks is relatively the same; (b). Each peak obtained is only one color; (c). peak height can vary by up to 3 times the difference between the highest peak and the lowest peak [9].

3.4. Analysis of PepYLCV APWS Sequencing and Bioinformatics Results
After obtaining information about the differences between the PepYLCV-APWS gene and the other isolates from West Sumatra, BLASTn PepYLCV-APWS was carried out on NCBI. This was done to identify the level of similarity between PepYLCV-APWS isolates and PepYLCV in Indonesia. Furthermore, ten different isolates were selected with a similarity percentage of about 85-99%. This level of similarity is identified based on the percentage value of identity (%) and Query Coverage (QC). In more detail, the level of similarity of PepYLCV can be seen in Table 1.

| No. | Accession Number | Identity (%) | QC (%) | Region            | Reference |
|-----|------------------|--------------|--------|-------------------|-----------|
| 1.  | LC.387328.1      | 99,7         | 99     | Aceh              | [10]      |
| 2.  | LC387327.1       | 99,6         | 99     | Aceh              | [10]      |
| 3.  | LC51112.1        | 89,8         | 88     | North Sumatera    | [11]      |
| 4.  | LC51113.1        | 90,8         | 88     | North Sumatera    | [11]      |
| 5.  | KT809346.1       | 91,9         | 90     | West Sumatera     | [4]       |
| 6.  | KT809345.1       | 92,4         | 89     | West Sumatera     | [5]       |
| 7.  | DQ83765.1        | 88,4         | 93     | West Java         | [12]      |
| 8.  | AB267836.1       | 88,2         | 93     | West Java         | [13]      |
| 9.  | AB267838.1       | 89,7         | 89     | West Java         | [13]      |
| 10. | LC54629.1        | 87,9         | 88     | Bali              | [14]      |

Based on table 1, PepYLCV-APWS have a similarity of 91.9% and query coverage 90% with an isolate from West Sumatera number accession KT809346.1. This isolate is known to be named PepYLCV-PSSWS which comes from Pesisir Selatan. PepYLCV-PSSWS is a very aggressive and virulent isolate, which is characterized by the appearance of symptoms on the 8th day after inoculation [5]. Meanwhile, the PepYLCV-APWS isolate had a similarity level with PepYLCV originating from Tanah Datar of 92.1% with a query coverage of 89%. Unlike the PepYLCV-PSSWS isolate, the PepYLCV-TDWS isolate showed symptoms in chili plants at the age of 21 after inoculation [4].

Based on the phylogenetic tree, some isolates are geographically located in the same area but different clades. This occurred in three isolates from West Sumatra, namely PepYLCV-APWS, TDWS, and PSSWS. This is because the PepYLCV-APWS isolate has changed the nucleotide sequence. This change is caused because the virus tends to always undergo evolution. According to [16] the virus will always mutate to maintain its life. The mutation ability of a virus is also influenced by its very simple genome structure, which is only DNA and RNA.
In Figure 4 it is suspected that recombination of the isolates between Pepylcv-APWS and PepYLVCVAV Bapep V4 originating from Aceh. This shows that these two isolates are in the same clade. This recombination will result in the molecular genetic diversity of PepYLCV. This information regarding the molecular diversity of Pepylcv-APWS is useful in implementing genome editing strategies, especially CRISPR, so that can be developed varieties of chili plants resistant to PepYLCV.

![Figure 4. Phylogeny Tree of PepYLCV](image)

4. Conclusion
From this research, it can be concluded as follows; the complete genome sequencing of Pepylcv APWS has been successfully carried out which the genome size is 2743 bp. The Pepylcv APWS is at the same clade as Pepylcv PSWWS but separated from Pepylcv TDWS which has been isolated in West Sumatra. For further research, it was suggested that Pepylcv APWS can be transformed into plasmid pBi121. Furthermore, a study on the promoter that plays a role in the APWS Pepylcv genome is suggested.

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