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# Molecular identification of the virus causing yellow curl symptoms in local Katokkon Chili Plants (*Capsicum chinense* Jacq.) in West Sulawesi

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***ABSTRACT***

*Katokkon Chili is an important local variety in Mamasa, West Sulawesi, which frequently exhibits yellow mosaic and yellow curl symptoms. These symptoms are strongly suspected to be associated with viral infections, particularly those in the genera Begomovirus and Potyvirus. This study aimed to molecularly identify viruses causing yellow curl symptoms in Katokkon Chili plants. Symptomatic leaf samples were collected purposively from 14 locations in Mamasa Regency, West Sulawesi, followed by molecular detection and identification using universal Begomovirus and Potyvirus primers and sequencing analysis. Detection and identification results showed that chili plants yielded 912 bp DNA fragments with SPG1/SPG2 primers, confirming viral identity as Pepper Yellow Leaf Curl Indonesia Virus (PYLCIV) and demonstrating >98% homology with PYLCIV isolates from South Sulawesi. This study reports the first occurrence of PYLCIV in the Katokkon Chili variety in West Sulawesi and expands the host range of PYLCIV among chili varieties in Indonesia. These findings are important as a foundation for developing viral disease control strategies for chili plants in West Sulawesi.*

***Keywords:***
*Begomovirus, Host range, Mamasa, PCR, Pepper yellow leaf curl Indonesia virus*

## 1. Introduction

Katokkon Chili pepper (*Capsicum chinense* Jacq.) is a leading local horticultural commodity that grows optimally in highland areas (1000–1500 m above sea level) and is cultivated in Mamasa Regency, Toraja, and the surrounding regions [1]. Katokkon Chili is known for its extremely high level of spiciness, distinctive aroma, abundant vitamin C and antioxidant content, stable price, and high production, as well as strong cultural value among the local community [2,3]. Its unique shape, adaptation to highland environments, and potential as a germplasm resource make Katokkon Chili an important innovative genetic source for chili breeding and national variety conservation programs [4,5].

The cultivation of Katokkon Chili can reach 14,000 to 50,000 plants per hectare, with a commonly used spacing of 50 × 40 am or 70 × 100 cm, and potential yields of up to 30 tons/hectare under optimal conditions [4,6], offering promising turnover and income potential for local farmers. However, the actual production and productivity of Katokkon Chili tend to be lower [7] than other chili varieties, ranging from 3.2 to 25.2 tons since 2022 [8]. The low production and productivity of Katokkon Chili may result from a combination of factors, including seed material quality, nutrient management, irrigation, and crop management practices, as well as pest and disease attack [9]. Common insect pests that attack chili plants include aphids (*Myzus persicae*, *Aphis gossypii*, *Bemisia tabaci*, *Aphis craccivora*), and *Thrips,* which are the main vectors

in the spread of viruses in chili plants [10]. Other commonly reported diseases in chili plants include *Fusarium wilt*, *Cercospora* sp., *Colletotrichum* sp., and virus infections [11,12].

Viral infections in chili plants are one of the main causes of decreased production and significant economic losses across Indonesia's main chili production centers. Early infection during the vegetative stage may result in up to 100% yield loss and is often more harmful than fungal or bacterial diseases [13–15]. Viral infections in chili plants can be caused by several different virus genera, including *Chili leaf curl virus* (ChiLCV) [16], *Pepper yellow leaf curl virus* (PYLCV) [17], *Pepper veinal mottle virus* (PVMV) [18], Tobacco mosaic virus (TMV) [19], *Cucumber mosaic virus* (CMV) [20], and *Tomato Spotted Wilt Virus* [21], which can be transmitted by insect vectors such as *Bemisia tabaci*, *Aphis gossypii*, and *Thrips* sp. [22–26]. In several regions in Indonesia, losses due to viral infection in chili are very large, with severe yield reductions occurring in susceptible Capsicum cultivars [27]. The magnitude of these losses depends on the timing of infection and host susceptibility, as these viruses are difficult to control and are generally systemic and spread by insect vectors, resulting in rapid and widespread transmission [10,20,28]. Some viruses are even capable of infecting plants simultaneously, known as mixed infections, and cause more complex symptoms and more severe damage [29,30]. Yellow mosaic or chlorosis, leaf curling, and leaf malformation are symptoms typical of viral infections associated with single or mixed *Begomovirus* and *Potyvirus* infections, and have been reported to infect most chili varieties (*C. annuum* L.) in Indonesia, including those in Java, Sumatra, Bali, and South Sulawesi [20,24,27,31,32].

Predominant viral infections have been widely reported in chili plants in Indonesia, and such symptoms have been frequently observed in Katokkon Chili in Mamasa Regency, West Sulawesi Province. To date, there have been no scientific reports regarding the presence of viruses infecting Katokkon Chili plants in West Sulawesi, especially concerning their molecular or phylogenetic identity. This situation highlights a significant gap in information, particularly since most virology studies on chili plants have so far focused on commercial varieties outside this area. Therefore, this research is designed as an initial report (first report) to detect the causal virus associated with yellow curl symptoms in Katokkon Chili from Mamasa Regency, West Sulawesi, and determine the genetic relationship through phylogenetic analysis with previously reported in Indonesia.

## 2. Methods

### *2.1. Time and Place of Research*

This research was conducted from July to September 2025. Samples of symptomatic Katokkon Chili were collected in Rantetarima Village and Saludengan Village, Bambang Subdistrict, Mamasa Regency, West Sulawesi Province. The preparation of extraction samples and molecular identification was carried out at the Molecular Laboratory, Integrated Laboratory Unit (UPT Laboratorium Terpadu), University of West Sulawesi.

### *2.2. Sample Collection*

Sample collection was based on fresh leaves of chili plants showing symptoms of viral infection, including yellow mosaic variations, leaf curling, green mosaic, dwarfing, leaf rolling upwards and/or downwards, and vein clearing at 14 different locations. Plant age was not systematically recorded in this survey. At each sampling point, 10 symptomatic plants were purposively sampled. The obtained leaf samples were stored in airtight plastic bags with ice packs in an ice box and the rest of sample stored in Falcon tubes containing silica gel for backup in the lab. Subsequently, the samples were stored in a freezer –80 °C before being used for DNA extraction and molecular analysis.

### *2.3. Molecular Identification with Polymerase Chain Reaction (PCR) and Reverse Transcription (RT)-PCR*

Molecular detection of viruses associated with yellow curl symptoms in Katokkon Chili leaves was conducted using PCR-based approaches, employing RT-PCR for Potyvirus detection and conventional PCR for Begomovirus. The workflow consisted of nucleic acid extraction, reverse transcription for RNA viruses, amplification of target genomic regions, and visualization of PCR products.

### *2.4. Total RNA Extraction*

Total RNA was isolated from symptomatic leaf tissue using a modified CTAB-based protocol as previously described [33], with minor adjustments. Approximately 0.1 g of leaf tissue was homogenized under cryogenic conditions and incubated in 500 µL CTAB extraction buffer (1% 2-β-mercaptoethanol) to facilitate cell lysis and RNA stabilization. The homogenate was transferred into a 2 ml microtube and incubated at 65 °C in a waterbath for 30min. The tube was inverted every 10 min to aid in the lysis. After 30 min of incubation, the plant extract tube was removed from the water bath and incubated further for 2 min at room temperature when 500 µL of Chloroform: Isoamyl alcohol mixture (24:1) was added. The supernatant was then pipetted into a clean tube and an equal volume of isopropanol added. The microtube was inverted until RNA threads were visible and then centrifuged at 12,000 rpm for 7 min. At the end of centrifugation, an RNA pellet was visible as the supernatant and isopropanol solution were poured off carefully from the RNA pellet. The RNA pellet was resuspended in 500 µL of 70% ethanol. Ethanol was added, after which the RNA pellet was spun down for 7 min at 12,000 rpm. Ethanol was then removed and the tube was inverted onto tissue for 15 min to allow air drying of the pellet. The resulting pellet was dissolved in 50 µL of 1 x TE (10 mM Tris-HCl pH 8, 0.1 mM EDTA).

**cDNA Synthesis.** First-strand cDNA synthesis was carried out using oligo(dT) primers and M-MuLV reverse transcriptase following the manufacturer's recommendations. The reverse transcription (RT) reaction mixture is composed of the following: 3.7 µL $dH_2O$, 0.50 µL 10 mM dNTPs, 0.75 µL oligo d(T), and 2 µL total RNA (veltus). The solution is gently mixed and incubated at 65 °C for 5 minutes and then cooled on ice immediately. Next, 2 µL of RT buffer, 0.50 µL of 10 mM dNTP, 0.35 µL of 50mM DTT, 0.35 MmuLv (Thermo Scientific), and RNase inhibitor (Thermo Scientific) in a final volume of 10µL were added to complete the reaction. The resulting cDNA was directly used as a template for PCR amplification.

**cDNA Amplification.** Viral cDNA amplification is carried out using universal *Potyvirus* primer pairs (Table 1) with an amplification program according to Table 2. The PCR reaction mix (25 µL) contains 12.5 µL GoTaq Green 2x (Thermo Scientific), 1 µL reverse primer (10µM), 1 µL forward primer (10µM), 9.5 µL nuclease-free water and 1ul cDNA.

**Total DNA Extraction.** Total RNA was isolated from symptomatic leaf tissue using a modified CTAB-based protocol as previously described [33], with minor adjustments. Approximately 0.1 g of leaf tissue was homogenized under cryogenic conditions and incubated in 500 µL CTAB extraction buffer (1% 2-β-mercaptoethanol) to facilitate cell lysis and RNA stabilization. The homogenate was transferred into a 2 ml microtube and incubated at 65 °C in a water bath for 30min. The tube was inverted every 10 min to aid in the lysis. After 30 min of incubation, the plant extract tube was removed from the water bath and incubated further for 2 min at room temperature when 500 µL of Chloroform: Isoamyl alcohol mixture (24:1) was added. The supernatant was then pipetted into a clean tube and an equal volume of isopropanol added. The microtube was inverted until RNA threads were visible and then centrifuged at 12,000 rpm for 7 min. At the end of centrifugation, an RNA pellet was visible as the supernatant and isopropanol solution were poured off carefully from the RNA pellet. The RNA pellet was resuspended in 500 µL of 70% ethanol. Ethanol was added, after which the RNA pellet was spun down for 7 min at 12,000 rpm. Ethanol was then removed and the tube was inverted onto tissue for 15 min to allow air drying of the pellet. The resulting pellet was dissolved in 50 µL of 1 x TE (10 mM Tris-HCl pH 8, 0.1 mM EDTA).

**DNA Amplification.** Viral DNA amplification is carried out using universal *Begomovirus* primer pairs as shown in Table 1, with the amplification program according to Table 2 [27]. The PCR reaction mix (25 µL) contains 12.5 µL GoTaq Green 2x (Thermo Scientific), 1 µL reverse primer (10 µM), 1 µL forward primer (10 µM), 9.5 µL nuclease-free water and 1 µL cDNA.

**Table 1. Primer sequences for amplification of viruses infecting chilli peppers**

| Primer[1] | Sequens (5′ ke 3′) | Product PCR (bp) | Ref. |
|---|---|---|---|
| *Potyvirus*-CIFor | GGIVVIGTIGGIWSIGGIAARTCIAC | 700 | [34] |
| *Potyvirus*-CIRev | ACICCRTTYTCDATDATRTTIGTIGC | | |
| SPG1 | CCCCKGTGCGWRAATCCAT | | |
| SPG2 | ATCCVAAYWTYCAGGGAGCTAA | 900 | [35] |

[1]F = *forward*; R = *reverse*

**Table 2. Amplification program for detecting *Potyvirus* and *Begomovirus* in chili peppers**

| Virus Target | PCR Programs | | | | | ∑ Cycle |
|---|---|---|---|---|---|---|
| | Pre-denaturation | Denaturation | Annealing | Elongation | Final Extention | |
| *Potyvirus* | 94 °C.3 min$^{-1}$ | 94 °C.1 min$^{-1}$ | 40 °C.30 sec$^{-1}$ | 68 °C.1 min$^{-1}$ | 68 °C.5 min$^{-1}$ | 40 |
| *Begomovirus* | 94 °C.5 min$^{-1}$ | 94 °C.1 min$^{-1}$ | 50 °C.1 min$^{-1}$ | 72 °C.1 min$^{-1}$ | 72 °C.7 min$^{-1}$ | 30 |

**DNA visualization.** DNA amplification was performed by 1% agarose gel electrophoresis. The gel was prepared by heating Agarose (0.3 g) and 30 ml of 0.5x

TBE buffer in a microwave for 2 minutes and allowed to mix well. Once the gel is warm, add 1.5 µl of FlouroVue TM nucleic acid dye (Smobio, Taiwan), mix thoroughly, then pour the agarose into a gel tray and let it sit for ± 30 minutes until it hardens. Electrophoresis is performed at a voltage of 50 volts for 50 minutes. The electrophoresis results in the form of DNA bands are visualized with an ultraviolet translumiluminator and documented with a digital camera.

**DNA Sequence Analysis.** Representative PCR amplicons were submitted for bidirectional nucleotide sequencing (1st Base, Malaysia). Obtained sequences were assembled and analyzed using BLASTn to identify homologous sequences available in the NCBI GenBank database. Multiple sequence alignments were performed using ClustalW implemented in BioEdit software. Phylogenetic relationships were inferred using the Neighbor-Joining method in MEGA version 11, applying the Tamura–Nei substitution model with 1,000 bootstrap replications to evaluate branch support. Nucleotide identities were computed using BLASTn and pairwise alignments; species demarcation followed the established threshold (>= ~89% for Begomovirus species).

## 3. Results and Discussion

### *3.1.* Variation of *Symptom*

Variations in symptoms in Katokkon Chili samples at 14 locations showed a spectrum of leaf damage in the form of yellow mosaic, chlorosis (yellowing of leaves), yellow-green mosaic, curling and cupping on the surface and edges of leaves, small leaves and leaf malformations, as well as vein clearing or vein banding. These symptoms are highly characteristic of viral infections from the *Begomovirus* and *Potyvirus* genera, particularly the *Pepper yellow leaf curl Indonesia virus* (PYLCIV), which has been consistently reported to cause similar symptoms in chili peppers across various regions of Indonesia, such as Bali and Java [22,27,36]. Single or multiple infections, between the *Begomovirus* genus and other viruses, have been reported to cause chili plants to become stunted and disease incidence to reach 100%, resulting in significant losses due to disruption of photosynthate distribution and plant metabolism [17,24,37].

Symptoms of yellow mosaic, vein banding, narrowing of leaf lamina, and severe malformation may appear in the event of mixed infection between several virus species from different genera, including, for example, *Chilli leaf curl virus* (ChiLCV), *Cucumber Mosaic Virus* (CMV), *Tobacco Mosaic Virus* (TMV), *Tomato spotted wilt virus* (TSWV), PVY, *Alfalfa mosaic virus* (AMV), *Pepper vein mottle virus* (PVMV), *Beet curl top virus* (BCTV), and *Pepper mild mottle virus* (PMMoV) [38,39]. Variations in symptoms between sampling locations can be influenced by virus type and strain, the genetic susceptibility of local varieties, plant age, and interactions between viruses and insect vectors such as *Bemisia tabaci* in *Begomovirus* and *Aphis gossypii* in *Potyvirus* [22,40]. Environmental conditions such as temperature, humidity, and soil fertility also influence the severity of symptom manifestation [41]. Visual symptoms are highly consistent with the dominant symptoms of plants infected with *Begomovirus* and *Potyvirus*.


**Figure 1. Planting conditions and symptom variations in Katokkon Chili plants in the field. (a) Yellowing accompanied by stunting; (b) V*ein clearing*; (c) V*ein banding*; (d) V*ein banding* accompanied by leaf malformation; (e) Green mosaic accompanied by leaf thickening; and (f) Yellow-green mosaic with stunting and leaf malformation**

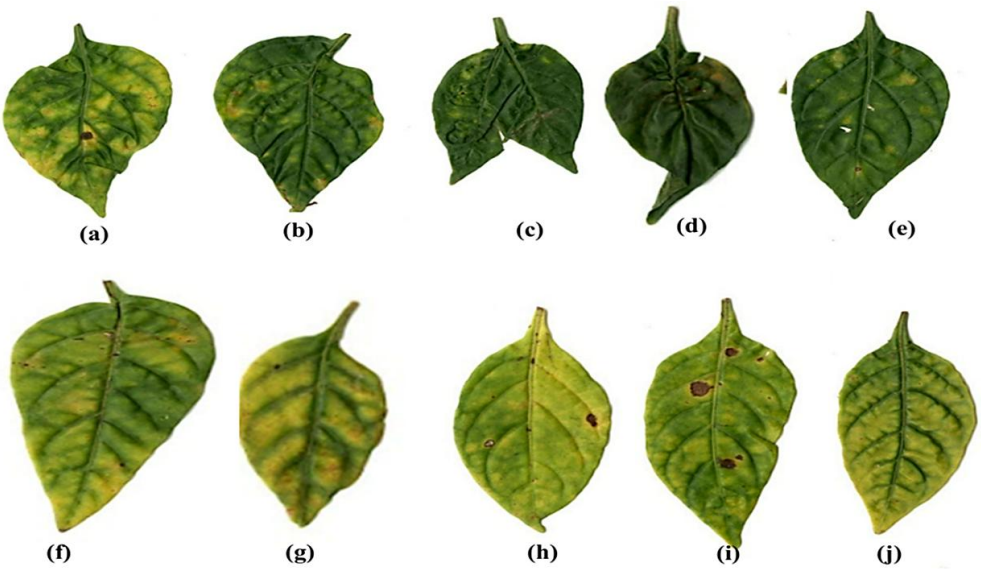


**Figure 2. Variations in dominant symptoms found at 14 sampling locations. (a-e) Curling, cupping, vein banding and vein clearing, yellow mosaic; (f-j) Yellow mosaic accompanied by vein banding and vein clearing, and necrosis**

### *3.2.* Molecular Identification of Viruses in Katokkon Chili Plants

A total of five composite symptomatic of Katokkon Chili leaves samples were tested by PCR/RT-PCR using *Begomovirus* and *Potyvirus* universal primers. Begomovirus was detected in five of five total samples (percentage = 100%) shows the amplicon approximately 912 bp (Figure 3a). In contrast, Potyvirus universal primer RT-PCR produced no detectable amplicons in five samples (Figure 1a). These results indicate that *Begomovirus* infection was predominant among the symptomatic plants we examined. This is consistent with several previous reports stating that the characteristic yellow curled leaves in chili peppers are more often associated with *Begomovirus* infection than *Potyvirus* [22,40].

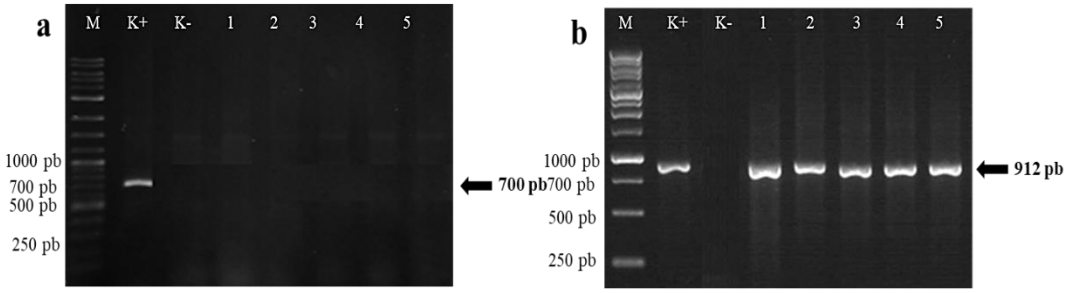


**Figure 3. RT-PCR visualization results using universal primers *Potyvirus* Cifor/Cirev: (a) and RT-PCR visualization results using universal primers *Begomovirus* SPG1/SPG2 (b); Composite samples of symptomatic Katokkon Chili leaves from West Sulawesi (1-5); K+. Positive control, K-. Negative control, M. DNA ladder 1 kb (Thermo Fisher Scientific, USA)**

Symptoms of chlorosis (yellowing leaves), yellow-green mosaic, curling and rolling on the surface and edges of leaves, small and malformed leaves, and vein clearing or vein banding in Katokkon Chili plants are caused by infection from *Begomovirus* [42]. The consistency of DNA fragment amplification in all samples confirms that the infection occurred uniformly and indicates the presence of a dominant virus in the chili pepper population at the study site.

*Potyvirus* was not found to infect Katokkon Chili samples from Mamasa. The failure to amplify Potyvirus with universal degenerate primers does not exclude the possibility of Potyvirus infection. Negative RT-PCR results may be due to low viral titer in sampled tissue, primer-template mismatches (local Potyvirus sequence divergence), or host resistance effects reducing virus accumulation. Fang et al. [43] revealed that the host range of *Potyvirus* is very specific depending on the variety and genotype of the host plant. In addition, the Katokkon variety (*C. chinense*) is reported to have a gene that is resistant (pvr1 gene) to several *Potyvirus* genera, including PepMoV, TEV, and PVY [44,45]. Environmental conditions and plant physiology can be factors that limit *Potyvirus* infection in Katokkon Chili varieties, including temperature, humidity, and plant age at the time of infection (latency period) [46]. The low concentration of *Potyvirus* in DNA and the incompatibility of universal primers with local *Potyvirus* strains may be the reason for the non-amplification of *Potyvirus* in the samples in this study. This is consistent with case study reports that negative PCR test results for *Potyvirus* may be due to resistant genotypes and incompatibility with the universal primers use [47].

The nucleotide sequence analysis was performed using the Basic Local Alignment Search Tool (BLAST) on the National Center for Biotechnology Information (NCBI) website, showing that the coat protein (CP) gene originating from the *Begomovirus*

**Table 3. Homology (%) of nucleotide sequences of PYLCIV isolates from West Sulawesi with several isolates already reported in GenBank**

| Sequence | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | ID | | | | | | | | | | | | | | | |
| 2 | 99% | ID | | | | | | | | | | | | | | |
| 3 | 99% | 99% | ID | | | | | | | | | | | | | |
| 4 | 99% | 99% | 99% | ID | | | | | | | | | | | | |
| 5 | 96% | 96% | 96% | 96% | ID | | | | | | | | | | | |
| 6 | 95% | 95% | 95% | 95% | 95% | ID | | | | | | | | | | |
| 7 | 95% | 95% | 94% | 95% | 94% | 95% | ID | | | | | | | | | |
| 8 | 94% | 94% | 95% | 94% | 93% | 94% | 98% | ID | | | | | | | | |
| 9 | 95% | 95% | 94% | 95% | 94% | 95% | 99% | 97% | ID | | | | | | | |
| 10 | 95% | 95% | 94% | 95% | 94% | 95% | 99% | 98% | 99% | ID | | | | | | |
| 11 | 95% | 95% | 94% | 95% | 94% | 95% | 99% | 97% | 99% | 99% | ID | | | | | |
| 12 | 93% | 93% | 93% | 93% | 93% | 96% | 94% | 93% | 94% | 94% | 94% | ID | | | | |
| 13 | 93% | 93% | 93% | 93% | 94% | 94% | 93% | 91% | 92% | 93% | 92% | 93% | ID | | | |
| 14 | 91% | 91% | 91% | 91% | 93% | 93% | 92% | 90% | 91% | 92% | 91% | 93% | 92% | ID | | |
| 15 | 91% | 91% | 91% | 91% | 93% | 93% | 92% | 90% | 91% | 92% | 91% | 93% | 92% | 99% | ID | |
| 16 | 44% | 44% | 45% | 45% | 44% | 45% | 44% | 44% | 44% | 44% | 44% | 44% | 44% | 43% | 43% | ID |

Note: 1. PYLCIV_Sulbar; 2. PQ899829_IDN_Gowa, 3. LC381263_IDN_Bali, 4. LC381264_IDN_Bali, 5. PP874899_IDN_YKT, 6. LC381265_IDN_Bali, 7. LC381261_IDN_Bali. 8. LC381277_IDN_Bali, 9. LC381279_IDN_Bali, 10.LC381280_IDN_Bali, 11. LC381270_IDN_Bali, 12. LC381259_IDN_Bali, 13. PQ539482_IDN_YKT, 14. LC547428_IDN_Bengkulu, 15. LC547427_IDN_Bengkulu dan 16. ToLCNDV_Nganjuk_LC311791 (outgroup isolate)

genus, with the identity of *Pepper yellow leaf curl Indonesia virus* (PYLCIV) was confirmed to infect Katokkon Chili plants originating from Mamasa, West Sulawesi, with a nucleotide homology value ranging from 91–99% against several isolates of Pepper yellow leaf curl Indonesia virus in the gene bank, including isolates from Bali, Bengkulu, Yogyakarta, and South Sulawesi (Table 3). The PYLCIV isolate from Mamasa, West Sulawesi, has the highest homology with the Gowa and Bali isolates (LC381263), followed by isolates from Yogyakarta, Bengkulu, and other Bali isolates from different hosts. The 99% homology value of PYLCIV with the Gowa and Bali isolates (LC381263) indicates the existence of a new PYLCIV host in chili varieties that are different from chili plant varieties in Indonesia and confirms the spread of PYLCIV in West Sulawesi. This is in line with the opinion of Fauquet et al. [48] that the *Begomovirus* genus, which has a nucleotide sequence homology value of ≥ 89%, can be classified into the same virus species. Given the confirmed presence of PYLCIV in Katokkon and the documented host range of PYLCIV across solanaceous hosts and weeds, plus the local presence of the whitefly vector *Bemisia tabaci*, PYLCIV represents a potential threat to other chili varieties grown nearby in West Sulawesi.

The phylogenetic tree revealed that the Mamasa isolate clustered together with PYLCIV isolates previously reported from Gowa and Bali, showing high nucleotide identity values (98–99%) and strong bootstrap support on major branches. Hamdayanty et al. [32] reported that PYLCV infection has infected red chili plants in Gowa Regency, South Sulawesi. The close relationship between the PYLCV isolate from South Sulawesi and the PYLCIV isolate from West Sulawesi may be due to their close geographical location. PYLCIV can be transmitted through several alternative hosts such as tomatoes, eggplants, cucumbers, and several weed species, such as *Ageratum* spp. and Ludwigia, and through the insect vector *Bemisia tabaci* [31,42]. The outgroup isolate used was the Tomato leaf curl New Delhi virus (ToLCNDV) isolate from Nganjuk as a comparison, which belongs to the genus *Begomovirus* and is distantly related to the PYLCIV isolate from West Sulawesi (Figure 4).

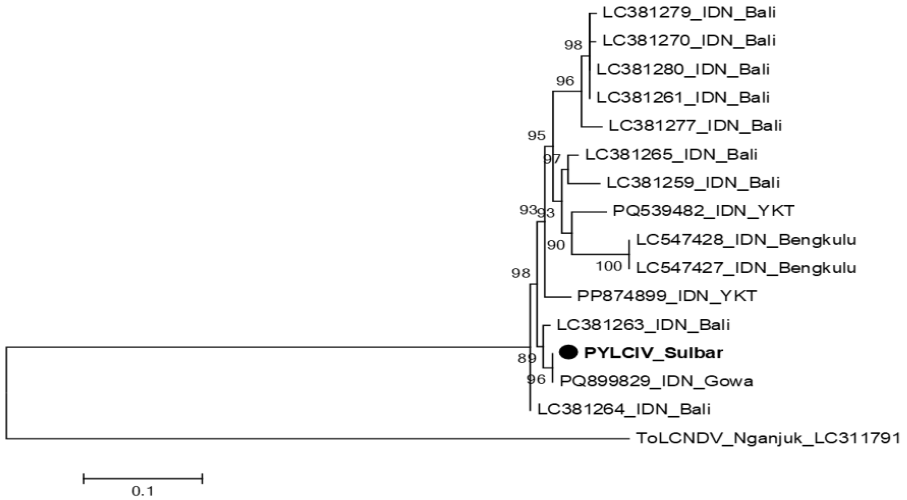


**Figure 4. Phylogenetic tree of *Pepper yellow leaf curl Indonesia virus* isolates from West Sulawesi**

## 4. Conclusion

This study reveals that *Pepper yellow leaf curl Indonesia virus* (PYLCIV) is the virus causing yellowing and curling symptoms in Katokkon Chili plants originating from Mamasa, Sulawesi, making it the first report and expanding the host range of PYLCIV, which has spread throughout West Sulawesi. This poses a threat to chili cultivation in West Sulawesi due to the availability of both insect vectors and alternative hosts for PYLCIV. The results of this study can be used as a reference in determining strategies to control the spread of PYLCIV in other chili plants in West Sulawesi.

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