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Molecular Identification and Genetic Analysis of the Virus Causing Yellow Leaf Disease in Eggplant (*Solanum melongena* L.) in Padang and Pariaman, West Sumatra

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Abstract

One of the major challenges in eggplant (*Solanum melongena* L.) production in Padang and Pariaman, West Sumatra, is the prevalence of yellow leaf disease, which is caused by plant viral infections. The incidence of this disease in eggplant crops in these regions reaches up to 90%, with a severity level of approximately 53.08%. This study aims to identify the causative virus of yellow leaf disease in eggplant plants in Padang and Pariaman and to determine the phylogenetic relationship of the virus with other yellow leaf-inducing viruses previously reported in the GenBank database. The research involved several stages: field observation and symptom description of yellowing leaves, viral RNA isolation, cDNA synthesis, DNA isolation, and DNA amplification (PCR) using specific primers. The CMV 1F/CMV 1R primers (650 bp) were used to detect viruses in the Cucumovirus group, MJ1/MJ2 primers (320 bp) for the Potyvirus group, and SPG1/SPG2 primers (912 bp) for the Geminivirus group. Genomic sequencing and phylogenetic tree analysis were then conducted to determine the genetic relationships of the identified virus. Field observations in Marunggi, Kuranji, and Sitoga villages identified eggplant plants showing typical symptoms of Geminiviridae infection. Molecular identification using PCR confirmed the presence of a geminivirus (912 bp) as the causative agent of the yellow leaf disease. Phylogenetic analysis revealed that the geminivirus identified in Padang is closely related to the *Tomato yellow leaf curl Kanchanaburi virus* (TYLCVKaV) from Thailand, with a nucleotide similarity of 95.30%. Nucleotide sequence characterization of surrounding weed species indicated the presence of a virus closely related to *Tomato yellow leaf curl New Delhi virus*, with a high similarity of 97.03%.The virus responsible for yellow leaf disease in eggplant plants in Padang and Pariaman, West Sumatra, was identified as a Geminivirus belonging to the family *Geminiviridae*. This virus is closely related to the *Tomato yellow leaf curl Kanchanaburi virus* (TYLCVKaV) from Thailand.

Keywords: Cucumovirus; Geminivirus; Potyvirus; phylogenetic tree

Introduction

Eggplant (*Solanum melongena* L.) is currently a lucrative crop in the agricultural sector in West Sumatra. Eggplant production can be disrupted by several factors, including pest attacks and plant diseases. One of the major challenges in eggplant production in Padang and Pariaman, West Sumatra, is the prevalence of yellow leaf

disease, which is caused by plant viral infections. The incidence of this disease in eggplant crops in these regions reaches up to 90%, with a severity level of approximately 53.08%.

Research Method

This research was conducted in Kuranji, Sitoga and Marunggi District. A total of five yellow leaf virus-infected samples were collected from Padang and another five from Pariaman, in addition to two weed samples located near eggplant crops in Pariaman. Tecnic of identification molecular using PCR. Step of identification molecular. (1) field observation and symptom description of yellowing leaves. (2) Viral RNA Isolation, (3) cDNA synthesis, (4) DNA isolation, (5) DNA amplification (PCR) using specific primers, analysis data. Primer (1) CMV 1F/CMV 1R primers (650 bp) were used to detect viruses in the Cucumovirus group, (2) MJ1/MJ2 primers (320 bp) for the Potyvirus group, (3) SPG1/SPG2 primers (912 bp) for the Geminivirus group.

Results and Discussion

A survey conducted in eggplant fields in Padang and Pariaman revealed that yellowing disease symptoms in eggplants included leaf malformation, leaf curling, mosaic patterns on leaves, chlorosis, vein banding, and vein clearing (Figure 1).

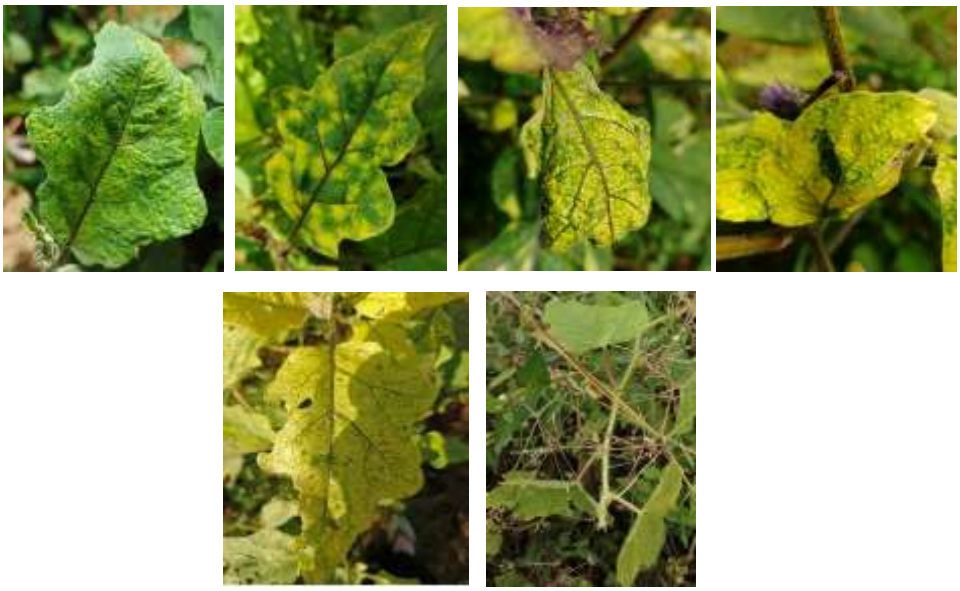


Figure 1. The symptom description of yellowing leaves in eggplant and weed in Padang and Pariaman

Eggplant samples exhibiting yellowing symptoms collected from the field were subjected to total RNA extraction using an RNA isolation kit at the Biotechnology Laboratory, Faculty of Agriculture, Universitas Andalas. The extracted total RNA was then used for cDNA synthesis of the causative virus via the polymerase chain reaction (PCR) method. The cDNA was amplified using RT-PCR with SPG primers, MJ primers, and CMV primers. Among the primers tested, only the Geminivirus-specific primers SPG1 and SPG2 successfully amplified the target fragment, yielding a DNA

fragment of approximately 912 bp from both Padang and Pariaman samples exhibiting yellowing symptoms (Figures 2–4).

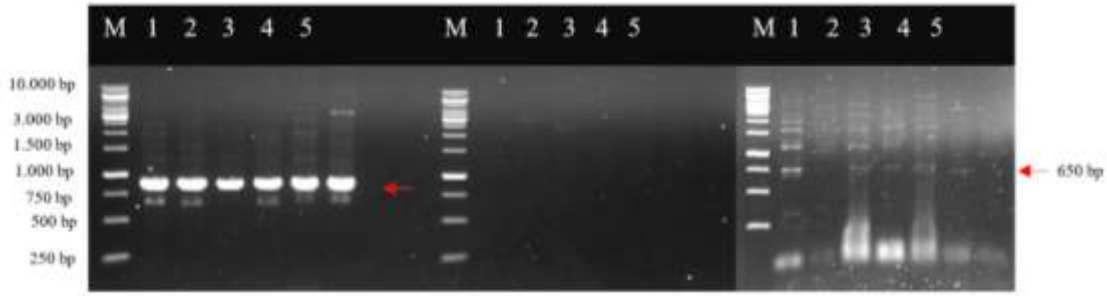


Figure 2. Visualization of cDNA amplification of eggplant-padang samples using SPG primers (left), MJ (center), CMV (right)

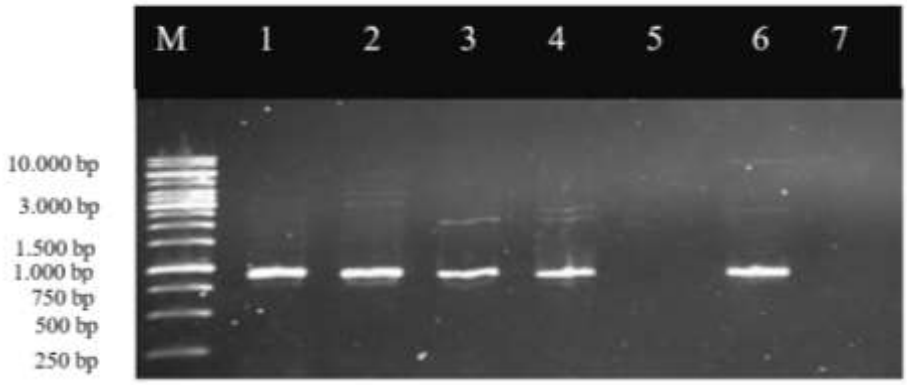


Figure 3. Visualization of cDNA amplification of eggplant-Pariaman samples using SPG Primers

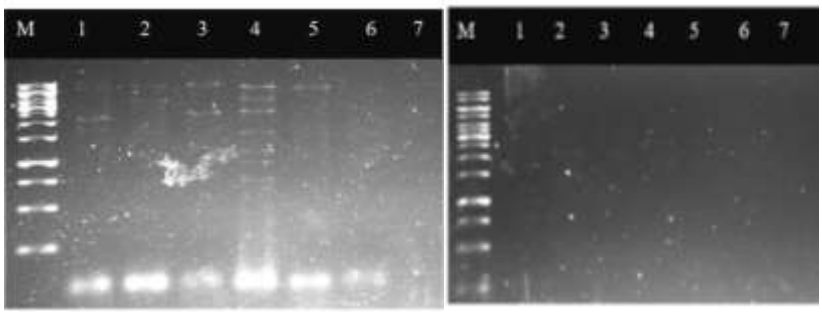
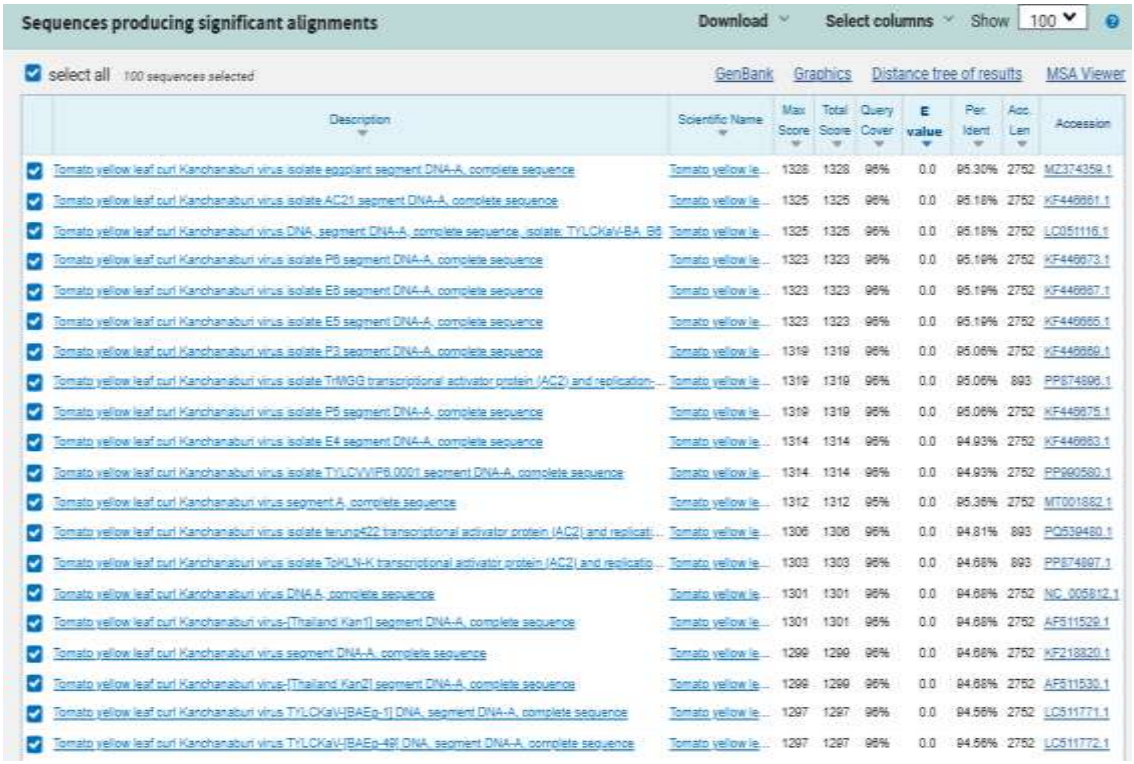


Figure 4. Visualization of cDNA amplification of eggplant-Pariaman samples using CMV Primers (left) and MJ Primers (right)

The amplified DNA was sequenced to analyze its molecular characteristics and to identify the causative virus. Nucleotide sequence similarity analysis of the virus associated with yellowing symptoms, compared to reference sequences in the GenBank database, revealed the highest similarity of 95.03% (Padang isolate 2) to *Tomato yellow leaf curl Kanchanaburi virus* (TYLCKaV) from Thailand (Figures 5–6). This finding is consistent with the report by Daulay and Sri (2020), who stated that yellowing disease eggplants is associated with TYLCKaV, a member of the *Begomovirus* genus.



Sequences producing significant alignments — Download — Select columns — Show 100

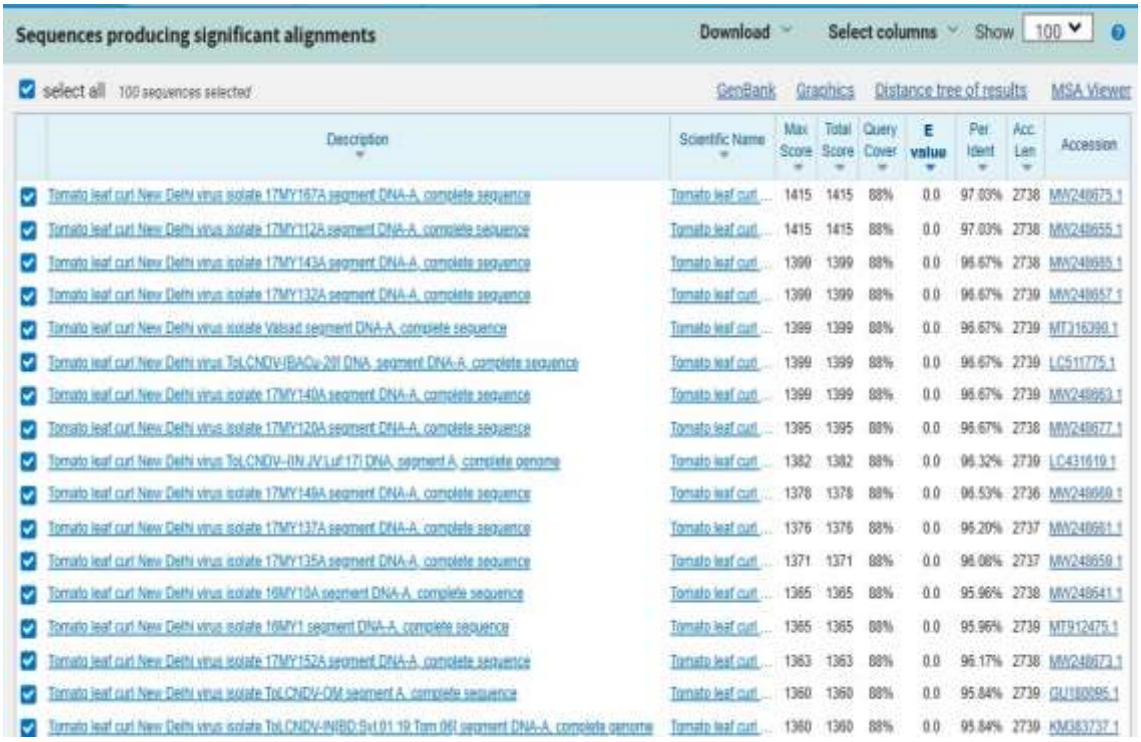
select all 100 sequences selected — GenBank — Graphics — Distance tree of results — MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Tomato yellow leaf curl Kanchanaburi virus isolate eggplant segment DNA-A, complete sequence	Tomato yellow le...	1328	1328	96%	0.0	95.30%	2752	MZ374359.1
Tomato yellow leaf curl Kanchanaburi virus isolate AC21 segment DNA-A, complete sequence	Tomato yellow le...	1325	1325	96%	0.0	95.18%	2752	KF446661.1
Tomato yellow leaf curl Kanchanaburi virus DNA, segment DNA-A, complete sequence, isolate: TYLCKaV-BA_B6	Tomato yellow le...	1325	1325	96%	0.0	95.18%	2752	LC051116.1
Tomato yellow leaf curl Kanchanaburi virus isolate P6 segment DNA-A, complete sequence	Tomato yellow le...	1323	1323	96%	0.0	95.18%	2752	KF446673.1
Tomato yellow leaf curl Kanchanaburi virus isolate E8 segment DNA-A, complete sequence	Tomato yellow le...	1323	1323	96%	0.0	95.18%	2752	KF446667.1
Tomato yellow leaf curl Kanchanaburi virus isolate E5 segment DNA-A, complete sequence	Tomato yellow le...	1323	1323	96%	0.0	95.18%	2752	KF446665.1
Tomato yellow leaf curl Kanchanaburi virus isolate P3 segment DNA-A, complete sequence	Tomato yellow le...	1319	1319	96%	0.0	95.06%	2752	KF446669.1
Tomato yellow leaf curl Kanchanaburi virus isolate TrMGG transcriptional activator protein (AC2) and replication-...	Tomato yellow le...	1319	1319	96%	0.0	95.06%	893	PP874896.1
Tomato yellow leaf curl Kanchanaburi virus isolate P5 segment DNA-A, complete sequence	Tomato yellow le...	1319	1319	96%	0.0	95.06%	2752	KF446675.1
Tomato yellow leaf curl Kanchanaburi virus isolate E4 segment DNA-A, complete sequence	Tomato yellow le...	1314	1314	96%	0.0	94.93%	2752	KF446663.1
Tomato yellow leaf curl Kanchanaburi virus isolate TYLCVVIP6.0001 segment DNA-A, complete sequence	Tomato yellow le...	1314	1314	96%	0.0	94.93%	2752	PP990580.1
Tomato yellow leaf curl Kanchanaburi virus segment A, complete sequence	Tomato yellow le...	1312	1312	95%	0.0	95.36%	2752	MT001882.1
Tomato yellow leaf curl Kanchanaburi virus isolate terung422 transcriptional activator protein (AC2) and replicati...	Tomato yellow le...	1306	1306	96%	0.0	94.81%	893	PQ539480.1
Tomato yellow leaf curl Kanchanaburi virus isolate ToKLN-K transcriptional activator protein (AC2) and replicatio...	Tomato yellow le...	1303	1303	96%	0.0	94.68%	893	PP874897.1
Tomato yellow leaf curl Kanchanaburi virus DNA-A, complete sequence	Tomato yellow le...	1301	1301	96%	0.0	94.68%	2752	NC_005812.1
Tomato yellow leaf curl Kanchanaburi virus-[Thailand Kan1] segment DNA-A, complete sequence	Tomato yellow le...	1301	1301	96%	0.0	94.68%	2752	AF511529.1
Tomato yellow leaf curl Kanchanaburi virus segment DNA-A, complete sequence	Tomato yellow le...	1299	1299	96%	0.0	94.68%	2752	KF218820.1
Tomato yellow leaf curl Kanchanaburi virus-[Thailand Kan2] segment DNA-A, complete sequence	Tomato yellow le...	1299	1299	96%	0.0	94.68%	2752	AF511530.1
Tomato yellow leaf curl Kanchanaburi virus TYLCKaV-[BAEp-1] DNA, segment DNA-A, complete sequence	Tomato yellow le...	1297	1297	96%	0.0	94.56%	2752	LC511771.1
Tomato yellow leaf curl Kanchanaburi virus TYLCKaV-[BAEp-49] DNA, segment DNA-A, complete sequence	Tomato yellow le...	1297	1297	96%	0.0	94.56%	2752	LC511772.1

Figure 5. The BLAST sequence data obtained from eggplant sample 2 collected in Padang

Figure 6. The phylogenetic tree constructed for eggplant sample 2 from Padang

Nucleotide sequence similarity analysis of the virus associated with yellowing symptoms in weed plants surrounding eggplant crops in Pariaman revealed the highest similarity of 97.03% (isolate B6 Ter B1) to *Tomato yellow leaf curl* New Delhi virus (Figures 7–8).



Sequences producing significant alignments — Download — Select columns — Show 100

select all 100 sequences selected — GenBank — Graphics — Distance tree of results — MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per Ident	Acc. Len	Accession
Tomato leaf curl New Delhi virus isolate 17MY167A segment DNA-A, complete sequence	Tomato leaf curl...	1415	1415	88%	0.0	97.03%	2738	MW248675.1
Tomato leaf curl New Delhi virus isolate 17MY112A segment DNA-A, complete sequence	Tomato leaf curl...	1415	1415	88%	0.0	97.03%	2738	MW248655.1
Tomato leaf curl New Delhi virus isolate 17MY143A segment DNA-A, complete sequence	Tomato leaf curl...	1399	1399	88%	0.0	96.67%	2738	MW248665.1
Tomato leaf curl New Delhi virus isolate 17MY132A segment DNA-A, complete sequence	Tomato leaf curl...	1399	1399	88%	0.0	96.67%	2739	MW248657.1
Tomato leaf curl New Delhi virus isolate Valsad segment DNA-A, complete sequence	Tomato leaf curl...	1399	1399	88%	0.0	96.67%	2739	MT316390.1
Tomato leaf curl New Delhi virus ToLCNDV-[BACu-20] DNA segment DNA-A, complete sequence	Tomato leaf curl...	1399	1399	88%	0.0	96.67%	2739	LC511775.1
Tomato leaf curl New Delhi virus isolate 17MY140A segment DNA-A, complete sequence	Tomato leaf curl...	1399	1399	88%	0.0	96.67%	2739	MW248663.1
Tomato leaf curl New Delhi virus isolate 17MY120A segment DNA-A, complete sequence	Tomato leaf curl...	1395	1395	88%	0.0	96.67%	2738	MW248677.1
Tomato leaf curl New Delhi virus ToLCNDV--[IN:JV:Luf:17] DNA, segment A, complete genome	Tomato leaf curl...	1382	1382	88%	0.0	96.32%	2739	LC431619.1
Tomato leaf curl New Delhi virus isolate 17MY149A segment DNA-A, complete sequence	Tomato leaf curl...	1378	1378	88%	0.0	96.53%	2736	MW248669.1
Tomato leaf curl New Delhi virus isolate 17MY137A segment DNA-A, complete sequence	Tomato leaf curl...	1376	1376	88%	0.0	96.20%	2737	MW248661.1
Tomato leaf curl New Delhi virus isolate 17MY135A segment DNA-A, complete sequence	Tomato leaf curl...	1371	1371	88%	0.0	96.08%	2737	MW248659.1
Tomato leaf curl New Delhi virus isolate 16MY10A segment DNA-A, complete sequence	Tomato leaf curl...	1365	1365	88%	0.0	95.96%	2738	MW248641.1
Tomato leaf curl New Delhi virus isolate 16MY1 segment DNA-A, complete sequence	Tomato leaf curl...	1365	1365	88%	0.0	95.96%	2739	MT912475.1
Tomato leaf curl New Delhi virus isolate 17MY152A segment DNA-A, complete sequence	Tomato leaf curl...	1363	1363	88%	0.0	96.17%	2738	MW248673.1
Tomato leaf curl New Delhi virus isolate ToLCNDV-OM segment A, complete sequence	Tomato leaf curl...	1360	1360	88%	0.0	95.84%	2739	GU180585.1
Tomato leaf curl New Delhi virus isolate ToLCNDV-IN[BD:Sv1:01:19:Tom:06] segment DNA-A, complete genome	Tomato leaf curl...	1360	1360	88%	0.0	95.84%	2739	KM383737.1

Figure 7. The BLAST sequence data obtained from eggplant sample B6 Ter B1 collected in Pariaman

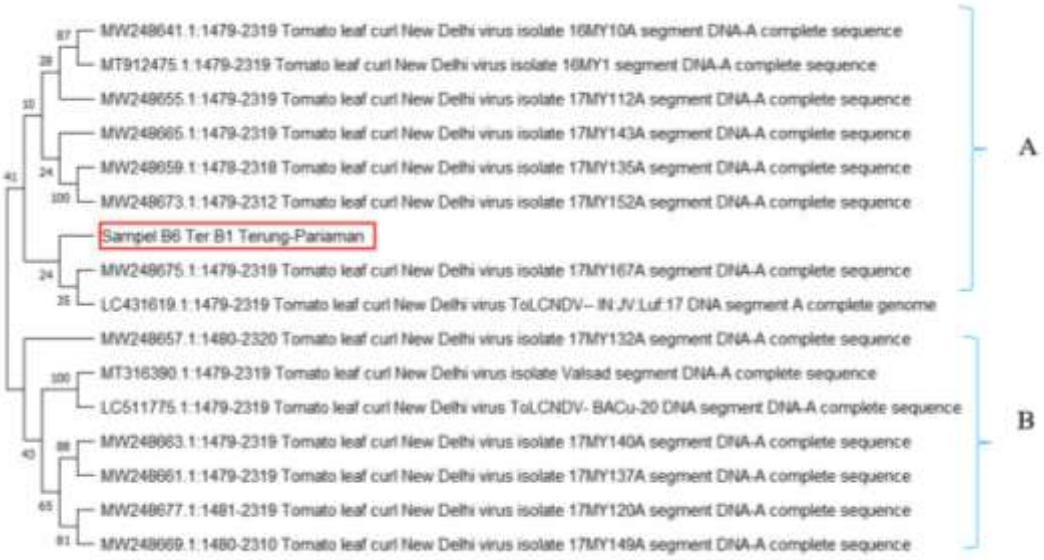


Figure 8. The phylogenetic tree constructed for eggplant sample B6 Ter B1 from Pariaman

Based on the results of detection and identification, mosaic and leaf curling symptoms (leaf malformation) as well as vein banding observed in eggplant were caused by TYLCKaV. In contrast, yellowing symptoms observed in surrounding weed plants were caused by TYLCV.

Conclusion

The conclusion of this study is: 1). The yellow leaf disease in eggplant plants in Padang and Pariaman, West Sumatra, was identified as a Geminivirus belonging to the family *Geminiviridae*; 2). This virus is closely related to the *Tomato yellow leaf curl Kanchanaburi virus* (TYLCKaV) from Thailand, with a nucleotide similarity of 95.30%; 3).Nucleotide sequence characterization of surrounding weed species indicated the presence of a virus closely related to *Tomato yellow leaf curl New Delhi virus*, with a high similarity of 97.03%.

Acknowledgement

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