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Detection of Gemini Virus on Tomato Plants (*Solanum lycopersicum* Mill.) in Karo North Sumatera Using Molecular Techniques

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ABSTRACT

Tomatoes are susceptible plants to various plant pests. One of the obstacles in tomato cultivation in Karo North Sumatera is the interference from various microorganisms such as plant virus attacks. The symptoms are the same as other diseases when in the field which causes difficulties in determining the type of virus that causes a disease, so it is necessary to carry out further testing as a good prevention. This study used the Polymorphism Chain Reaction (PCR) technique with universal primers for the Gemini virus. All samples of diseased tomato leaves originating from the Karo were attacked by Geminivirus which correlated with the presence of a DNA band measuring 1.5 kb after amplified.
Keyword: Karo North Sumatera, *Solanum lycopersicum* Mill., Gemini virus

ABSTRAK

Penelitian ini menganalisis keragaman dan kelimpahan plankton serta benthos di Sungai Bahorok, Bukit Lawang, Kabupaten Langkat, Provinsi Sumatera Utara. Penelitian ini dilaksanakan pada tanggal 25 Mei 2024. Pengambilan sampel dengan cara pengumpulan air yang disaring menggunakan plankton net dan substrat menggunakan surber net untuk pengambilan benthos dari dua titik di sungai. Identifikasi plankton dan benthos menggunakan mikroskop serta analisis laboratorium untuk parameter fisikokimia. Nilai keanekaragaman plankton di Sungai Bahorok terindeks baik dengan nilai 3,01 begitu juga dengan kemerataan yang baik dengan nilai 0,95 namun, keanekaragaman benthos terindeks rendah dengan nilai 0,68 dan kemerataan terindeks sedang dengan nilai 0,62. Jenis plankton tertinggi yaitu Raphidiopsis raciborskii. Jenis benthos tertinggi yakni Progomphus sp.
Keyword: *Kelimpahan, Benthos, Plankton, Komposisi struktur*

1. Introduction

Tomato (*Solanum lycopersicum* Mill.) is a shrubs of the Solanaceae family [1]. Tomatoes are one of the agricultural commodities that have high demand in Indonesia markets. The average annual demand for tomatoes is 20%. The increasing demand for tomatoes is due to an increase in population by 1.8% per year and per capita consumption growth increasing by 17.3% [2].

Indonesia has several of the largest tomato producing regions, one of which is North Sumatera. This province is in the second position as the largest tomato producer in Indonesia. Climate and soil conditions which is good for supporting tomato cultivation to develop well in North Sumatera. According to Badan Pusat Statistik [3], North Sumatera tomato production in 2023 is 2,038,676 tons. One of the areas in North Sumatera which is the highest center for tomato production in Karo Regency in Karo, North Sumatera tends to experience a decline starting from 1,639 ,007 tonnes in 2021, and 1,326,849 tonnes in 2022. However, tomato crop production in 2023 is 1,424,025 tonnes, but still lower than in 2021 One of the obstacles in tomato cultivation in Karo North Sumatra is interference from various microorganisms such as pest attacks. Symptoms that often seen are yellow leaves on the interveinal side, cupping leaves, rough and harden texture [4]. Gemini virus (Begomovirus) from the Geminiviridae family is a pest that has a circular DNA genome and has a single strand [5]. Begomovirus can be carried by whitefly vector insect (Bemisia tabaci) which has a wide host range and infect dicot plants so that its distribution is quite wide [6]. Disease symptoms in tomatoes are difficult to detect morphologically, because there are similarities in the symptoms caused by various viruses. so more specific disease detection is needed. Polymerase Chain Reaction (PCR) technique is the most widely used to detect and identify viruses. It is because this technique is very specific, quick and accurate. This research can be useful for the community as information about tomato disease in Karo, North Sumatra. So the result can be used as a comparative data to control plant diseases and making superior seeds.

2. Materials and Methods

2.1 Sample collection

Tomato leaves with virus symptoms are taken from Karo. The samples were taken randomly selected from six districts. They are Barus Jahe, Berastagi, Dolat Rakyat, Kabanjahe, Merdeka and Simpang Empat. The samples that were taken belong to symptoms of chlorosis leaves, rough and thick leaf texture, curly and cupping leaf, and healthy leaves as negative control.

2.2 DNA isolation

DNA isolation was carried out using the Cetyl Trimethyl Ammonium Bromide (CTAB) method by Doyle and Doyle [7]. The samples were crushed 0,1 g and put into a mortar containing 700 µL of 2% CTAB extraction buffer (20 mM EDTA, 0,1 M Tris-HCl pH 8, 1,4 M NaCl, 2% CTAB, and 0,4% β-Mercaptoethanol) which have been pre-heated at 65 ºC, then crushed using a pestle. The fine sample was put into a 1,5 mL microtube and incubated at 65 ºC for 45 minutes, then added 500 µL of phenol- chlorophome-isoamyl alcohol (25:24:1) and vortexed. Samples were centrifuged at 12.000 rpm for 10 minutes. The supernatant was transferred to a new tube and 500 µL of phenol-chlorophome-isoamyl alcohol (25:24:1) was added. The supernatant was transferred to a new tube and added 700 µL of cold isopropanol, then incubated in the freezer for 2 hours. Samples were centrifuged at 12.000 rpm for 10 minutes. The pellets were washed with 500 µL of 70% alcohol and centrifuged at 12.000 rpm for 10 minutes and dried. The pellets were dissolved with 50 µL of aquabides, incubated for 1 hour at 37 ºC and stored at -20 ºC as stock.

2.3 Electrophoresis

The samples visualized by electrophoresis on 1% agarosa in 1X TAE, using 100 Volt voltage for 45 minutes. The agarose gel was soaked in Ethidium Bromide (EtBr) for 10 minutes. The agarose gel that has been soaked in aquabides for 5 minutes. The gel was visualized under a UV transilluminator and documented on a documentation gel.

2.4 Quantitative DNA Test Using Nanofotometer

The genomic DNA that has been obtained is tested using a nanofotometer to see the concentration and purity of the sample DNA. A total of 1 µL of aquabides was dropped into the nanofotometer cuvette. The lid is placed on the cuvette and the blank button is pressed for calibration. Then 1 µL of sample DNA was dropped into the nanofotometer cuvette and the sample button was pressed. The purity of the sample DNA was measured at the wavelength A260 / 280.

2.5 PCR analysis

DNA amplification using the PCR method used universal primers for the Gemini virus, namely PAL1v 1978 and PAR1c 715 [8]. The sequences of primers can be seen in **Table 1**. Composition of PCR reactans can be seen in **Table 2**. PCR programe for detecting Gemini virus in tomatoes can be seesn in **Table 3**.

Table 1. Primary sequences for detection of Gemini virus in tomatoes

Components	Sequences	PCR
PAL1v 1978-F	5'-GCATCTGCAGGCCCACATYGTCTTYCCNGT-3'	1500
PAR1c 715-R	5'-GATTTCTGCAGTTDAATRTTYTCRTCCATCCA-3'	bp

F = *Forward*
R = *Reverse*

Table 1. Composition of PCR reactants

Components	Volume (µl)
Nuclease Free Water	9,5
Go Taq Green Mix	12,5
Primer Forward	1,0
Primer Reverse	1,0
DNA	1,0
Total	25

Target	PCR Conditions (°C/ min)					Cycle
	Pre-denaturatio	Denaturatio n	Annealing	Elongation	Final Extension	
Gemini Virus	94/ 4	94/ 1	52/ 1	72/ 2	72/ 1	30

2.6 Electrophoresis of DNA Amplification Results

The results of DNA amplification were analyzed by electrophoresis on 1,5% agarose gel in 1X TAE using 70 Volt voltage for 30 minutes. The agarose gel was soaked in Ethidium Bromide (EtBr) for 10 minutes. The agarose gel that has been soaked in aquabides for 5 minutes. The gel was visualized under a UV transilluminator and documented on a documentation gel.

3. Results and Disccusion

3.1 Morphological Symptoms of Tomato Leaves Infected Gemini Virus

A survey conducted on tomato plants infected with the Gemini virus from 5 sub districts in 18 villages in Karo North Sumatera showed that tomato plants in each village had symptoms due to Gemini virus attacks, that is curly and stiff leaves, curled leaf edges, thickened leaf bones, the leaves's color is yellowish green to bright yellow, there are mosaics and malformations on the leaves. This symptom is a common symptom of most virus infected leaves. The comparison of healthy plants with plants infected virus can be seen in **Figure 1**.

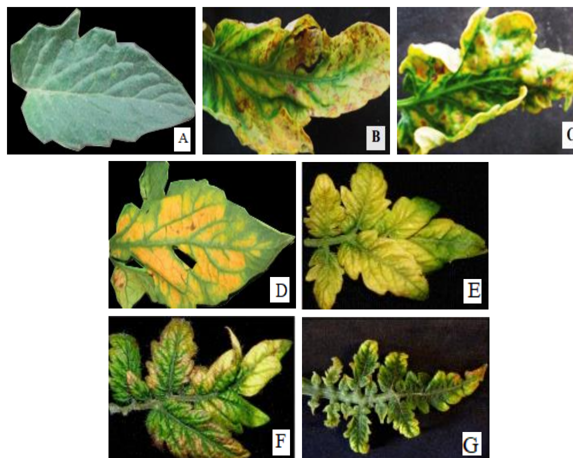

Figure 1. Symptoms variation in tomato leaves infected Gemini virus: healthy leaves (A), chlorosis between leaf bones, brown spots and upward curved leaf edges (B), chlorosis between leaf bones, curly leaves, thick and necrotic (C), there is a mosaic on the leaves (D), yellowish leaf color (E), hard and stiff leaf bones (F), smaller leaf size (G).

Dominant symptoms in diseased tomato plants are curly and stiff leaves, leaf edges roll upwards, and yellowish green to bright yellow leaf color. The least symptom seen is the reduced leaf size **Table. 4**. Mosaic is an irregular difference in green and yellow colors in the plant canopy, especially the leaves [9] . Mosaic symptoms are found in the villages of Barus Julu, Tanjung Barus, Rumah Rih, Sempa Jaya, Suria Indah, Tongkoh, Beganding, Kacaribu, Kandibata, Guru Lion, Spirit and Gajah Village. Symptoms of reduced leaf size are found in the villages of Barus Julu, Tanjung Barus, Rumah Rih, Sempa Jaya, Suria Indah, Tongkoh, Ujung Sampun, Beganding, Kacaribu, Kandibata, Pribadi and Gajah.

Table 4. Symptoms identified in four districts constituting 18 villages in Karo.

	(district, village)	*Curly and stiff leaves*	*Leaf Malformation*	*Ring mosaics*	*Leaf edges curl up*	*Thickened leaf bones*	*Reduced leaf size*	*The color of the leaves is yellowish green to bright yellow*	*Vein clearing*
1.	*Barus julu (S1)*	√	√	√	√	√		√	
2.	*Tanjung Barus (S2)*	√	√	√	√			√	√
3.	*Rumah Rih (S3)*	√	√	√	√		√	√	√
B.	Berastagi								
4.	*Lau Gendek (S4)*	√			√				
5.	*Sempa Jaya (S5)*	√					√		√
6.	*Raya (S6)*			√				√	
7.	*Suria Indah (S7)*		√			√	√		√
C.	Dolat Rakyat								
8.	*Tongkoh (S8)*		√	√			√		
9.	*Ujung Sampun (S9)*	√	√			√		√	
D.	Kabanjahe								
10.	*Beganding (S10)*				√	√			
11.	*Kacaribu (S11)*	√		√					
12.	*Kandibata (S12)*			√				√	
E.	Merdeka								
13.	*Guru Singa (13)*							√	√
14.	*Semangat (14)*		√		√	√			
F.	Simpang Empat								
15.	*Bulan Baru (S15)*						√	√	√
16.	*Gajah (S16)*	√	√	√		√		√	
17.	*Peteguhan (S17)*				√		√		√
18.	*Ujung Simalem (S18)*	√		√		√			√

Symptoms of Gemini virus infection in plants generally include thickening of the veins of the leaves, curling of the leaf edges up and down, yellowing of the leaves, and their size becoming small. Symptoms of virus infection in the field are the same and vary because the expression of symptoms is influenced by the type of virus, environmental conditions and plant cultivar [10]. The occurrence of curling in leaves is caused by excessive growth of cells or cell tissue on one side or local growth in cells on one side without being followed

by cell growth on the other side. This is related to the production of the hormone auxin which functions as a regulator of cell elongation and cell differentiation. The variations in the symptoms that appear can be caused by environmental influences. In addition, the attack of pathogens on a certain type of plant shows different reactions, that is between very vulnerable and very resistant [11].

3.2 The Results of DNA Isolation

Genomic DNA was isolated from tomato leaves with virus symptoms and electrophoresed on 1% agarose. The results of DNA isolation can be seen in Figure 2.

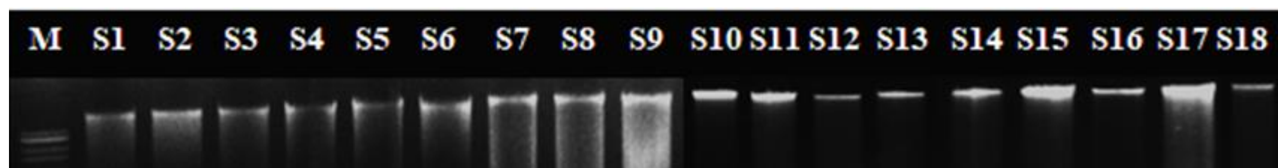

Figure 2. Genomic DNA bands from 18 samples of tomato leaves electrophoresed using 100 Volt voltage for 45 minutes (M = Marker 1 kb, S1-S18 = DNA bands of virus infected tomato plants).

The results of DNA isolation of virus stricken tomato plants showed quite clear DNA bands. However, the DNA bands obtained have different thicknesses. This is influenced by physical treatment at the time of extraction. In addition, differences in DNA purity can also be caused by residual materials such as loading dye in the agarose gel pores, so that there is a thin smear between the DNA bands.

The states that thick and clumped DNA bands (not spread) show high concentrations and the extracted total DNA is intact. Meanwhile, the DNA strands that appear to be spreading show that the bonds between DNA molecules are broken during the extraction process, so that the DNA genome is cut into smaller pieces. The breaking of the bonds between these molecules can be caused by excessive physical movement in the pipetting process, when turned back and forth, centrifuged, too high temperatures or due to the activity of certain chemicals [12].

The difference in band thickness obtained in each sample can be due to the buffer's ability to extract and the physical treatment it provides. The process of physical destruction of cells can facilitate the extraction buffer to break down cells and can determine the quality of the DNA produced. Overall the results of the tomato plant DNA isolation have good quality, this is indicated by the presence of a clearly visible band so that it can be used for the next stage [13].

3.3 DNA Quantitative Test Results

The results of the purity of the DNA of tomato plants infected the virus showed that the DNA had been isolated had good purity, which was free from protein and RNA contaminants (Table 5.). The difference in the results of the quantity can be caused by the technicality at the time of measurement, part of the DNA attached to the tube and the improper pipetting process caused the DNA to break into fragments. These technical errors caused the DNA concentration in the nanofotometer results to be less than the DNA quality test results. One of the reasons for the lack of extracted DNA and the purity that is not close to 100% is the technical aspect of the implementation of each stage carried out, such as when separating the supernatant from its sediment at the stage of removing protein residues and other materials that may be taken too little so that the weighted DNA weight is not taken up and the amount of DNA taken is small. Incomplete drying of the alcohol can also cause contamination which has an effect on the quantity of DNA. High DNA purity and not contaminated by protein residues was indicated by the ratio A260 /280, namely 1,8 to 2,0. Results that show purity values below 1,8 indicate protein contamination. Meanwhile, the results that show purity above 2,0 indicate the presence of small molecular weight compound contaminants such as RNA, so it needs to be purified with the addition of RNAse. Impure DNA can also be caused by ethanol residue during drying. In addition, the residual content of secondary metabolites in extracted plant organs can also cause impure DNA [14].

Table 5. Concentration and Purity of genomic DNA of virus-infected tomato plants

No	Samples	DNA Concentration (ng/µl)	DNA purity (A260/280)
1	Sample 1	4.790	1.916
2	Sample 2	2.810	1.925
3	Sample 3	1.070	1.845
4	Sample 4	2.080	1.944
5	Sample 5	1.450	1.756
6	Sample 6	2.860	1.781
7	Sample 7	2.230	1.875
8	Sample 8	1.780	1.751
9	Sample 9	3.840	1.765
10	Sample 10	4.700	1.820
11	Sample 11	4.430	1.752
12	Sample 12	2.570	1.810
13	Sample 13	1.170	1.800
14	Sample 14	1.320	1.784
15	Sample 15	8.001	1.941
16	Sample 16	4.159	1.916
17	Sample 17	8.260	1.806
18	Sample 18	6.550	1.944

3.4 PCR Analysis Results

The results of DNA amplification of virus infected tomato plants by PCR using PAL1v1978-PAR1c715 primers showed that size of the DNA amplicon formed was 1.500 bp (Figure 3.). These results indicate that all samples (18) detected the presence of the Gemini virus. The 1500 bp DNA fragment is the Gemini virus DNA fragment. Several studies have reported the successful amplification used the same primers, namely PAL1v1978 and PAR1c715 to detect Gemini virus in tomato plants and the resulting amplification fragments measuring 1.500 bp [15;16].

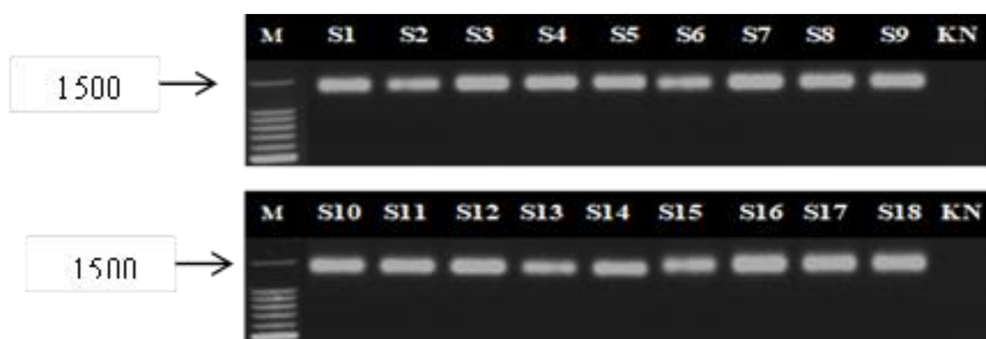

Figure 3. Electrophoresis of DNA amplification results from virus infected tomato plants using PAL1v1978-PAR1c715 primer on 1,5% agarose. (M = Marker 100 bp, S1-S18 = virus infected tomato DNA, KN = negative control).

The success of the PCR technique in detecting Gemini virus in plants and insect vectors shows that this technique can be used as part of a disease control strategy, especially for use in determining early warnings in the field. Several studies have reported the successful in detecting and identifying the disease caused by the virus using the PCR technique [17;18;19].

PCR technique depends on the concentration and purity of the sample DNA, Taq polymerase, primer length, primer composition and the level of primer homology with the target DNA so these factors must be controlled properly. In the annealing process, the spesific primer will attach to the DNA strands that have

separated into single strands. The primer will form a hydrogen bridge with the DNA strand in the sequence area that is complementary to the primer sequence [19].

The success of the PCR technique in detecting gemini virus in plants and insect vectors shows that this technique can be used as part of a disease control strategy, especially for use in determining early warnings in the field. The results of this research show that the disease caused by the gemini virus has spread in Karo Regency, North Sumatera.

4. Conclusion

In conclusion, all samples of diseased tomato leaves originating from Karo North Sumatera were attacked by the Gemini virus which was indicated by the presence of a DNA band measuring 1,5 kb (1500 bp) after amplification using spesific primers..

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