Begomovirus Detection On Diseased Chili Plant (*Capsicum annum* L.) In Tanah Karo North Sumatera With PCR Techniques

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**Abstract.** Begomovirus (from the Geminivirus) is one of the most common virus that causes losses of chili plants in Tanah Karo. The existence of Begomovirus infection effect the chili plant reduce the production, and make a decrease in both the quality and quantity of that. Begomovirus detection only by looking at morphological symptoms could not provide accurate results. It is because Begomovirus has symptoms that are almost identical to other viral diseases. Therefore this research was done to detect Begomovirus molecularly by using PCR technique. A total of 20 leaf samples from chili plant with symptomatic virus were taken from 20 villages in Tanah Karo. Results of the research were morphological symptoms such as vein clearing, thickened and curved leaf edges roll up (cupping), young leaves shrink and bright yellow colored leaves. PCR by using specific primers Begomovirus indicated the presence of a 1500 Bp DNA fragment in all samples of chili DNA. Anatomical results in the form of stomatal density showed healthy chili plants have greater stomata density compared to Begomovirus infected plants.

1. Introduction
Tanah Karo is one of the central area of chili farming in North Sumatra. Based on the recognition from chili farmers that chili production in Tanah Karo has decreased because of the crop failure and can not reached the target of demand. The causal factor is plant diseases that infect chili plants including diseases caused by viruses. Symptoms of the disease are mosaic, bleaching, leaf shrinkage, vein clearing, thickened and curved leaf bones (cupping). Viral infections such as yellow curly leaves on chili plants were first reported in 1999 in West Jawa, an it caused by *Begomovirus* from the genus Geminivirus [1] and has spread rapidly into central chili plants in Indonesia [2]. Symptoms of this disease are very typical such as necrosis, mosaic, vein clearing and bleaching that not only found in chili plants but from other diseased plant.

So far, *Begomovirus* detection is mostly done by conventional methods by looking at the typical symptoms of chili plants that are attacked by disease. But this method has not been able to ensure that these symptoms are caused by *Begomovirus* or other viruses [3]. Therefore, a precise method is needed to detect the presence of *Begomovirus* in order to differentiate between symptoms of *Begomovirus* in chili plants. Moreover, accurate virus detection techniques become a requirement in the early stages of disease-causing diagnosis. The most commonly used detection techniques today are molecular detection techniques such as PCR (Polymerase Chain Reaction) that utilize the specific nature of virus nucleotide sequences. Detection by PCR has been widely carried out and provides accurate results. As done who succeeded in detecting MYMIV species by using the universal primary pairs PAL1v1978 / PAR1c715 [4], was successfully identified Tomato Yellow Leaf Curl Kanchanaburi Virus on eggplant plants using SPG1/ SPG2 primer [5]. PCR detection of viruses
provides accurate, fast and very sensitive results. The PCR technique only requires a bit of fresh, dried or frozen samples [6].

2. Methods
2.1 Sample Collection
Plant samples analyzed in the form of diseased chili plant leaves were chosen randomly from twenty area in Tanah Karo. The twenty areas were located in Dolat Rakyat, Barus Jahe, Nama Tern and Berastagi districts. One of each healthy and diseased plants were sampled from each areas.

2.2 DNA Extraction
Extraction was carried out using a method by Wylie et al., 1993 [7]. As much as 1 gram of leaf samples were crushed and 300 ml of extraction buffer was added (Tris-HCl 0.1 M, pH 8.0; EDTA 0.05 M; 0.5 M NaCl, 2-mercaptoethanol 0.01 M) and 500 μl PCI (Phenol Chloroform Iosamyl Alcohol) (24: 23: 1) then centrifuged at 13000x g for 5 minutes. The supernatant was added by CI (Chloroform Iosaaryl Alcohol) (50: 1) over 1 volume then centrifuged for 5 minutes at 13000x g. The supernatant then added with cold isopropanol and incubated for 1 night. Then centrifuged at 13000x g for 3 minute. The pellets then dissolved with 100 μl TE 10x, 4 μl NaCl 5M and 250 μl ethanol 96% and incubated for 15 minutes, then centrifuged again for 3 minutes at 13000x g. Pellets are washed with 70% cold ethanol and dried. After drying, the pellets were suspended in 50 μL of RNase-free water and stored at -20 oC to avoid damage so it can be used for next process.

2.3 DNA Amplification
Viral DNA amplification was carried out by the PCR method using Begomovirus-specific primary pairs (pAL1v1978-cpF=5'-GCATCTGAGGGCCCACATYGGTCTTTYCCNGT-3' and pAR1c715-cpR = 5'–GATTTCTGAGGTATATTTTCATCCA-3')[8]. The mixture and condition of amplification were set (Table 1, Table 2).

Table 1. The composition of the polymerase chain reaction (PCR) reactant for one time viral genome DNA amplification reaction.

| Components                  | Volume (μl) |
|------------------------------|-------------|
| RNase free water            | 9.5         |
| Go Taq Green Master Mix 2x (Thermo) | 12.5       |
| Primer R 10 μM              | 1.0         |
| Primer F 10 μM              | 1.0         |
| DNA                         | 1.0         |
| Total                       | 25          |

Table 2. PCR condition for detecting viruses in chili plants.

| Target     | PCR condition (°C/min) |
|------------|------------------------|
|            | Predenaturation | Denaturation | Annealing | Elongation | Final Extension | Cycle |
| Begomovirus | 94/4             | 94/1         | 52/1      | 72/2       | 72/1            | 30    |

2.4 Data Analysis
The results of DNA amplification then analyzed by electrophoresis on agarose with 1% concentration at 100 A for 30 minutes in 1% TAE buffer (pH 8). Staining of agarose gel using 10 mg / ml of Etidium bromide for 15 minutes then destaining with distilled water for 3 minutes. The amplified tape was observed using UV Transiluminator and saw the presence or absence of a specific band with the Begomovirus gene.

3. Result and Discussion
3.1 Viral Symptoms of Begomovirus
Surveys on chili plants infected with viruses from 20 rurals in Tanah Karo, North Sumatra (Table 3) show that chili plants in each rurals have symptoms due to virus attacks, that were the presence of a vein clearing, bleaching, thickened and curved leaf bones (cupping), young leaves shrink, mosaics and leaf malformations (Figure 1).

These symptoms were common found in most leaves of plants that have been infected with viruses, such as PYLCV, TMV and CMV. Some viruses that mostly attack chili plants in Indonesia such as CVMV (Chile Veinal Mottle Potyvirus), CMV (Cucumovirus Cucumber Mosaic), PMMV (Mild Mottle Potyvirus Peppers), and PYLCV (Yellow Leaf Curl Begomovirus Peppers) [9].

Figure 1. Comparison symptoms of chili plant leaves attacked by viruses: vein clearing (A), curly and stiff leaves (B), thickened leaf bones (C), mosaic (D), curve leaf bones (E), leaf size smaller (F), yellowish green leaf color (G) bright yellow (bleaching) (H) and leaf malformation (I) with healthy leaf control (J).
| No | District         | Village           | Vein clearing | Thickened and curve leaf | Leave shrinks | Malformation | Cupping | Small size | Bright yellow colored leaves | Mosaics | Jumlah |
|----|-----------------|-------------------|---------------|--------------------------|---------------|--------------|---------|------------|-------------------------------|---------|--------|
| 1. | Dolat Rayat     | Bukit (S1)        |               |                          |               |              |         |            |                               |         | 2      |
| 2. | Dolat Rayat     | Sampun (S2)       | √             |                          |               |              |         | √          |                               |         | 3      |
| 3. | Dolat Rayat     | Ujung Sampun (S3) |               |                          |               |              |         | √          |                               |         | 3      |
| 4. | Dolat Rayat     | Melas (S4)        |               |                          |               |              |         | √          |                               |         | 3      |
| 5. | Dolat Rayat     | Dolat Rayat (S5)  | √             |                          |               |              |         | √          |                               |         | 3      |
| 6. | Barus Jahe      | Barus Julu (S6)   |               |                          |               |              |         | √          |                               |         | 2      |
| 7. | Barus Jahe      | Bulan Jahe (S7)   |               |                          |               |              |         | √          |                               |         | 4      |
| 8. | Barus Jahe      | Bulan Julu (S8)   |               |                          |               |              |         | √          |                               |         | 3      |
| 9. | Barus Jahe      | Paribun (S9)      |               |                          |               |              |         | √          |                               |         | 3      |
| 10.| Barus Jahe      | Sukajulu (S10)    |               |                          |               |              |         | √          |                               |         | 2      |
| 11.| Berastagi       | Raya (S11)        |               |                          |               |              |         | √          |                               |         | 2      |
| 12.| Berastagi       | Guru Singa (S12)  |               |                          |               |              |         | √          |                               |         | 1      |
| 13.| Berastagi       | Gundaling II (S13)|               |                          |               |              |         | √          |                               |         | 3      |
| 14.| Berastagi       | Gundaling I (S14) |               |                          |               |              |         | √          |                               |         | 3      |
| 15.| Berastagi       | Doulu (S15)       |               |                          |               |              |         | √          |                               |         | 3      |
| 16.| Nama Teran      | Kuta Mbelin (S16) |               |                          |               |              |         | √          |                               |         | 5      |
| 17.| Nama Teran      | Kebayaken (S17)   |               |                          |               |              |         | √          |                               |         | 3      |
| 18.| Nama Teran      | Bekerah (S18)     |               |                          |               |              |         | √          |                               |         | 4      |
| 19.| Nama Teran      | Kuta Gunung (S19) |               |                          |               |              |         | √          |                               |         | 2      |
| 20.| Nama Teran      | Kuta Rayat (S20)  |               |                          |               |              |         | √          |                               |         | 4      |
|    | Health control  |                   |               |                          |               |              |         |            |                               |         |        |
| Jumlah |           |                   |   7          |    7                     |    8          |    6        |    9     |    5       |    6                           |    9    | 57     |
From Table 3. Can be seen that the most variable symptom due to viruses is found in chili leaf samples from Kuta Mbelin Village and Nama Teran District, while the least variation of symptoms due to viruses is found in chili leaf samples from Barus Julu and Barus Jahe District. The more dominant symptom found in infected chili plants is the leaf edges roll up (cupping) and there are mosaics. Mosaics are irregular differences in green and yellow colors in plant canopy, especially in leaves, while cupping is a symptom of small stiff leaves and curved edges upward. Symptoms in the form of cupping are not only found in chili plants, but in some plants attacked by viruses from the Geminivirus group such as TYLCV (Tomato Yellow Leaf Curl Virus) and Bean common mosaic virus (BCMV) [10]. The different variations of symptoms that occur in chili plants infected with the virus are usually caused by environmental factors such as the level of soil fertility and climate around plants, plant factors such as age and plant genotypes [11].

3.2 DNA Isolation
The leaves of the symptomatic chili plant were isolated using the method [7] was then electrophoresed on 1% agarose by setting an electric current of 100 A for 30 minutes (Figure 2). The results of the DNA total isolation of chilli plants showed DNA bands that were quite clear and could be used for the next process. The clearly DNA bands and are expected to have Begomovirus DNA in it, because the isolated leaf are leaf that are symptomatic of viral diseases.

![Figure 2](image_url)

**Figure 2.** DNA bands from 20 samples of chili plant leafes that electrophoresed by setting an electric current of 100 A for 30 minutes (S1-S20 = chili plant DNA bands infected with the virus).

* Begomovirus is a plant virus with genetic material in the form of circular single strands, so to obtain Begomovirus DNA isolation needs to be done on the leaf of chili plants that are symptomatic of disease [12].

3.3 Molecular Detection of Begomovirus from Chili Plant
Total DNA isolated from 20 leaf of symptomatic chili plants from Tanah Karo was used as a template for detecting disease in the chili plants. The presence of 1500 bp DNA fragments showed that all chili leaves (20 samples) which symptomatic of the virus from Tanah Karo positively contained Begomovirus (Figure 3). This is confirmed by the presence of the same band in the positive control in the form of Begomovirus DNA clones (Macrogen) and the absence of bands in the negative control which are in the form of healthy chili leaves.
Figure 3. Electrophoresis of DNA results from viral PCR symptomatic chili plants from Tanah Karo using Begomovirus specific primers on 1% agarose. (M = Marker; KN1 = Negative Control 1; KN2 = Negative Control 2; KP = Positive Control). a.) S1-S10 = chili plant DNA samples infected with the virus; b.) S11-S20 = chili plant DNA samples infected with the virus.

The primer also used (pAL1v1978 / pAR1c715) to detect Begomovirus virus in chili plants and produce 1500 bp fragments [2,6]. Begomovirus has nucleic acid in the form of single strand DNA, so that DNA can be directly used as a template (mold) in PCR [8]. Positive Begomoviruses in all symptomatic chili leaf samples prove that chili plants have been attacked by Begomovirus and have also been spread in villages in Tanah Karo. The spread of the virus is probably due to the fact that chili farmers use chili seeds from the same place and have been infected with the virus, and also because of the location of the chili fields, allowing the vector to carrying the virus easily move from one field to another.

4. Conclusion
Research on chilli plants infected with viruses in 20 villages in Tanah Karo, North Sumatra, showed symptoms of viral infection, namely a vein clearing on leaflets, thickened leaf bones, curved leaf edges (cupping), and further symptoms in young leaves, small size, bright yellow or light green leaflets with bright yellow, mosaics and malformations on the leaves. The results of PCR electrophoresis using a Begomovirus specific primer, pAL1v1978 / pAR1c715 amplified the 1500 bp DNA fragments in all samples. This shows that all chili plants are positive attacked by Begomovirus.

Acknowledgments
Detection needs to be done by using some virus-specific primers which attack chili plants in Tanah Karo to find out if there is a type of disease caused by another virus of chili plants in Tanah Karo, North Sumatra.
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