Detection of potato virus Y infecting potato leaves in Tanah Karo, North Sumatera, Indonesia using Reverse Transcriptase–Polymerase Chain Reaction (RT-PCR)

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Abstract. Accurate identification of plant pathogens is crucial towards developing sustainable control strategies to ensure sustainable economic in agricultural production. The aim of this study was to detect Potato virus Y (PVY) infecting potato (Solanum tuberosum L.) in the Tanah Karo district, province of North Sumatera, Indonesia. Potato leaf samples exhibiting virus-like symptoms were collected from twenty different areas in the district. Initial detection of PVY in the leaf samples was done using reverse transcription-polymerase chain reaction (RT-PCR) by primers specific to the coat protein gene of PVY. Virus-like symptoms were collected from twenty samples consisted of vein clearing, faint mosaic patterns, and the vein necrosis. All of potato leaf samples were detected to be infected by PVY. The widespread presence of PVY in Tanah Karo needs serious implications on the management of PVY diseases by small-scale farmers growing potato for a livelihood.

1. Introduction

Tanah Karo is central area of potato farming in Sumatera, Indonesia. However, the production of potatoes in this area has not reached the target of demand, even its production tends to decrease. One causal factor is plant diseases that infect potato plants, including diseases caused by viruses. Symptoms of viral infections in potato plants, such as necrosis, mosaic, chlorosis, green thickening veins, bleaching, striping, leaf shrinkage, to plant stunting have been identified by several different viruses, potato virus Y/ PVY (Potyviridae; Potyvirus), potato virus X/ PVX (Alphaflexiviridae, Potexvirus), and potato virus S/ PVS (Betaflexiviridae; Carlavirus) [1,2,3]. One of the most common diseases in potato plants is mosaic disease. Mosaic disease is an important disease because it can decrease the quality and quantity of potato production. Mosaic disease can be caused by Potato virus Y (PVY) [4].

Potato virus Y (Potyviridae: Potyvirus) is a virus that is of concern to the global attention because its attack on potato crops causes significant losses. The losses are the decrease of tuber yield in quality and quantity [5,6,7]. But the symptoms caused by the virus is difficult to be distinguished visually or directly in situ. Therefore, a precise method is needed to detect the presence of PVY virus in order to differentiate between symptoms of PVY among potatoes. Moreover, accurate virus detection techniques become a requirement in the early stages of disease-causing diagnosis [8]. The basic technique that has long been done to detect and identify plant viruses is through the observation
of virus particles using electron microscopy, symptoms observation on infected plants, host range tests, and viral transmission tests. Advances in detection technology has allowed the virus to be easily distinguished from other viruses by serological and molecular tests.

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. PCR techniques have been commonly used to detect viruses, such as tobacco leaf cracker virus, Chilli veinal mottle potyvirus (ChiVMV) in chili, Tomato viral chlorosis (ToCV) and Tomato infectious chlorosis virus (TiCV) [9,10,11]. The study then will reveal the prevalence of PVY infection in several potato farms in Tanah Karo with emphasize of symptoms relationship and clustering among study sites.

2. Materials and Methods

2.1 Sample Collection

Samples of potato plants exhibiting viral diseases from its leaves were chosen randomly from twenty rural area in Tanah Karo. The twenty rural areas were located in Dolat Rakyat, Barus Jahe, Tiga Panah, and Simpang Empat districts. One of each healthy and diseased plants were sampled from each rural areas.

2.2 Total RNA Extraction

The method used to extract total RNA was based on Wylie [12]. Diseased samples were crushed 0.1 g in 500 μL extraction buffer (Tris-HCl 50 mM, pH 8.5, EDTA 10 mM, NaCl 200 mM) and 500 μL phenol-chloroform-isooamyloalcohol (PCI). Mixture were homogenized and centrifuged at 13000x g for 1 min. Supernatant were added with 1 volume of chloroform-isooamyloalcohol (CI) (50:1) followed by centrifugation at 13000x g for 1 min. The supernatant were added with 1 volume of isopropanol, and incubated in -20 °C overnight. The supernatant were centrifuged at 13000x g for 3 mins. Pellets were dissolved by adding 100 μL TE buffer (Tris-HCl 10 mM, pH 7.4, EDTA 1 mM), 4 μL 5M NaCl, and 250 μL etanol 96%, followed by incubation in cold condition for 20 mins. Suspension were later centrifuged again at 13000x g for 3 mins to obtain pellets. Pellets were air-dried and kept in 50 μL RNase free water for further experiments.

2.3 cDNA Synthesis

The method used to synthesis cDNA was based on [4]. The complementary DNA synthesis (cDNA) is the reverse transcription process of viral RNA to cDNA by using Reverse transcription (RT) technique. Reagents such as 2 μl H2O, 0.5 μl dNTP 10 mM, 1 μl Oligo d (T) 10 mM, and 3 μl RNA template were reacted at 65 °C for 5 mins and immediately cooled in ice. The reagents were reacted again by adding 2 μl 5 × buffer RT, 0.5 μl DTT 0.1 M, 0.5 μl M-MuLV and 0.5 μl ribolock with proper mixing and incubated at 42 °C for 60 min and 70 °C for 10 min to inactivate enzyme. The RT result of cDNA is used as a DNA template in PCR reaction.

2.4 DNA Amplification

Viral DNA amplification was conducted using RT-PCR method by incorporating primers (PVY-cpF 5’-ATGGSAATGACACAACTGATGCA-3’ and PVY-cpR 5’-ACATGTTSACTCAAAGYG-3’) into PCR mixture to locate, amplify and detect PVY gene fragments (801 basepairs). The mixture and condition of amplification were set (Table 1; Table 2).
Table 1. PCR mixture composition for one cycle of amplification

| 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         |
| Cdna                              | 1.0         |
| Total                             | 25          |

Table 2. PCR conditions

| Target | Pre-denaturation | Denaturation | Annealing | Elongation | Final Extension | Cycle |
|--------|-----------------|--------------|-----------|------------|-----------------|-------|
| PVY    | 94/ 2           | 94/ 1        | 52/ 1     | 72/ 2      | 72/ 7           | 35    |

2.5 Data Analysis

The RT-PCR results were then visualized by electrophoresis on 1% agarose gel in a TAE 1 × buffer, using a 70 Volt voltage for 45 minutes. Gel soaked in ethidium bromide for 5 minutes. Gel agarose that has been soaked in sterile water for 10 minutes, then visualized under UV illumination inside gel doc.

3. Results and Discussions

3.1 Viral Symptoms of Potato Plants in Tanah Karo

Diseased potato leaves with symptoms of viral infection were sampled from twenty cultivation lands in Tanah Karo covering 4 districts, namely Dolat Rakyat, Barus Jahe, Tiga Panah and Simpang Empat. Symptoms of occurring viral infection were identified and categorized as light mosaics, ring mosaics, the difference in growth between the veins (rugose), veins clearing, veins banding, shortened stem segment, leaf distortion and even leaf malformation (Figure 1; Table 3). [13] stated that common external symptoms caused by virus attacks or infections on potato plants can be seen through changes in colors that are irregularly distributed.

Figure 1. Symptoms categories: (A) light mosaics, (B) ring mosaics, (C) stunting stem segment, (D) leaf distortion, (E) rugose, (F) vein clearing, (G) leaf malformation, (H) vein banding; control (I) healthy plant.
Table 3. Symptoms identified in four districts constituting 20 villages in Tanah Karo

| Location (District, Village) | Light Mosaics | Ring Mosaics | Stunting Stem Segment | Leaf Distortion | Ragose | Vein Clearing | Leaf Malformation | Leaf Malformation | Vein Banding |
|-----------------------------|---------------|--------------|-----------------------|-----------------|-------|--------------|------------------|------------------|-------------|
| **A. Dolat Rakyat**         |               |              |                       |                 |       |              |                  |                  |             |
| 1. Dolat Rakyat             | √             | √            |                       |                 |       |              |                  |                  |             |
| 2. Melas                    |               |              |                       |                 |       |              |                  |                  |             |
| 3. Sampun                   |               |              |                       |                 |       |              |                  |                  |             |
| 4. Ujung Sampun             |               |              |                       |                 |       |              |                  |                  |             |
| **B. Barus Jahe**           |               |              |                       |                 |       |              |                  |                  |             |
| 5. Persadanta               |               |              |                       |                 |       |              |                  |                  |             |
| 6. Barus Julu               |               |              |                       |                 |       |              |                  |                  |             |
| 7. Bulan Julu               |               |              |                       |                 |       |              |                  |                  |             |
| 8. Paribun                  |               |              |                       |                 |       |              |                  |                  |             |
| 9. Rumamis                  |               |              |                       |                 |       |              |                  |                  |             |
| **C. Tiga Panah**           |               |              |                       |                 |       |              |                  |                  |             |
| 10. Aji Jahe                |               |              |                       |                 |       |              |                  |                  |             |
| 11. Aji Jula                |               |              |                       |                 |       |              |                  |                  |             |
| 12. Aji Bahara              |               |              |                       |                 |       |              |                  |                  |             |
| 13. Bunuraya                |               |              |                       |                 |       |              |                  |                  |             |
| 14. Kuta Bale               |               |              |                       |                 |       |              |                  |                  |             |
| **D. Simpang Empat**        |               |              |                       |                 |       |              |                  |                  |             |
| 15. Beganding               |               |              |                       |                 |       |              |                  |                  |             |
| 16. Berastepu               |               |              |                       |                 |       |              |                  |                  |             |
| 17. Gajah                   |               |              |                       |                 |       |              |                  |                  |             |
| 18. Bulan Baru              |               |              |                       |                 |       |              |                  |                  |             |
| 19. Gamber                  |               |              |                       |                 |       |              |                  |                  |             |
| 20. Kuta Tengah             |               |              |                       |                 |       |              |                  |                  |             |

From the table, it can be seen that Dolat Rakyat was symptomized with leaf mosaics, leaf malformation and shortened stem segment. In Simpang Empat, the symptoms were leaf mosaics, vein banding and vein clearing (leaf bone). Mosaic symptoms are characterized by the presence of vein banding on potato leaf while in Indonesia most are caused by PVY [1]. Only two districts showed the most various symptoms, which were Barus Jahe and Tiga Panah. As for all variations of these symptoms are light mosaics, ring mosaics, rugose, vein clearing, vein appeal, shortened stem segment, leaf distortion and even leaf malformation. Symptom of viral infections that are commonly found in Tanah Karo is leaf mosaics. Mosaic is an irregular coloring of green and yellow of plants, especially on leaves [14]. However, the mosaic symptoms not only appeared in potatoes but also in other crops, such as Brassicaceae which is caused by Turnip Mosaic Virus (TuMV), eggplant plants by Bean Common Mosaic Virus (BCMV), soybean by Soybean Stunt Virus (SSV) and Soybean Mosaic Virus (SMV) [15,16,17].

Variation of symptoms of virus-infected plants is influenced by several factors, including age, of cultivars, plant genotypes, plant growth phase and environmental factors such as soil fertility and climate, so that these factors resulted in the decrease of quality and quantity of potato tubers. Based on this study, damaged tuber is characterized by spots forming on potatoes, more wavy surfaces and low-yielding tuber weights. This is because viral infections interfere with the physiological processes of potato plants [18,19]. Physiological abnormalities due to prolong of viral infections may be spotted from morphology of plants such as irregularly distributed colors and abnormal shapes and sizes on the...
leaves, stems, and fruits [13,20]. Prevalences of grouping symptoms occurring between villages are presented in Figure 2.

![Figure 2. Clustering of PVY symptoms among villages in Tanah Karo](image)

Twenty villages were clustered into different populations based on the similarity of PVY symptoms. The highest symptom similarities in the population was between district of Dolat Rakyat (Dolat Rakyat, Sampun village) and district of Simpang Empat (Gajah, Kuta Tengah, Berastepu, Bulan Baru, Beganding, Gamber village) with percentage of 100% (1.0). Organisms within a population share many similar characteristics or traits and relationships between their relatives [21]. Observation of symptoms conducted in Tanah Karo is the first step in the diagnosis of viral disease. Variation of symptoms found in several villages in Tanah Karo were related to characteristics of viral infections caused by PVY. However, the observation of symptoms alone is not accurate enough to determine precisely the presence of the virus. Because symptoms may be caused by other factors such as: other phytopathogens, insect vectors and the abiotic factors such as nutrient excess or deficiency, environmental stress and so on [14]. In addition, diseases of the plant can be infected by more than one kind of virus, as well as the one virus can cause multiple symptoms, therefore virus detection by using molecular technique like RT-PCR is required to confirm the presence of viral infection or PVY.

### 3.2 Molecular detection of PVY from potato plants

RNA from healthy plants and symptomatic leaves of viral infection sampled from 20 villages in Tanah Karo, was successfully amplified as shown by relatively good RNA bands (Figure 3) without any contaminants. RNA isolation is a technique for detecting diseases such as viruses. From the total RNA yielded from diseased plants, some thick RNA bands were formed. This is because the purity of RNA in the samples are higher than the others. Some authors stated technical issues regarding the resulting bands or fragments which were not visible or very thin, were caused by low concentration of RNA and the thick fragments were caused by high RNA concentrations while some RNAs with high impurities will produce smear band images [22,23]. RT-PCR amplification run successfully depending on the specific primary design and the purity and concentration of the DNA. Purity and concentration of DNA affects the intensity of amplified DNA bands on each primer [24]. Application of RT-PCR in this study produced of a successful viral RNA amplification at the size of about 801 base pairs (Figure...
4), and also proved that existing infection of potato plants in Tanah Karo is caused by Potato virus Y (PVY).

![Figure 3. Total RNA from infected potato plants](image1)

![Figure 4. Amplicons of RT-PCR from infected samples. Lane M = DNA Markers 100 base pairs, Lane k-1 = negative control (ddH2O), Lane k-2 = negative control (healthy plant), Lane 1 – 20 = Samples](image2)

The primary pairs used in the RT-PCR technique are universal primer pair of Potyviruses that have been used frequently for the purpose of detection and identification of viruses from the Potyvirus group. Several studies have reported the successful amplification of viral gene in Potyvirus group using specific primers such as PVY cp-F / PVY cp-R primers and PVYCPvBamH1 / PVYCPeEcoR1 primers, which are specially designed to amplify and detect the PVY virus of the same size of about 801pb [3,25]. With the detection of PVY virus infection of potato plants in Tanah Karo, it then may reflect the importance of proper management and disease prevention in order to control the infection in the future. We suggest the use of sequencing technique to ensure the identity of PVY infecting the potato plants as well as further investigations like testing the presence of other members of Potyvirus or other viruses that may infect the potato plants in Tanah Karo.

4. Conclusions

Samples of infected plant leaves taken from twenty villages in four districts of Tanah Karo showed typical symptoms of infection caused by Potato Virus Y (PVY) with various symptoms occurred.
Molecular technique (RT-PCR) is a useful diagnostic method to identify the presence of PVY among all infected samples by using universal primer pairs. All samples were positively infected by PVY, as visualized in electrophoresis by the presence of DNA bands sizing of 801 basepairs in all lanes.

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