Detection of virus causes papaya ringspot virus - with the DAS-Elisa (Double Antibody Sandwich-Enzyme-Linked Immunosorbent Assay) method at different levels in North Sumatra

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Abstract. This study aimed to detect the papaya ringspot virus (PRSV) in papaya plants in papaya plantations in North Sumatra with the Double Antibody Sandwich-Enzyme-Linked Immunosorbent Assay (DAS-ELISA) method. Research activities began with a survey to several locations of papaya plantations by observing the symptoms of PRSV-P, and carrying out virus detection with DAS-ELISA. The results showed that there were several types of symptoms of PRSV-P disease, namely ring-shaped spots; stripes, mosaics and leaf malformations; stripes symptoms; dark green streaks on stems and stems.

1. Introduction
Papaya (Carica papaya L.) is a plant that is cultivated in tropical countries including Indonesia [1]. According to data from the Central Statistics Agency, papaya fruit production in North Sumatra in 2014 decreased compared to 2013, namely in 2013 papaya fruit production was 27,757 tons to 26,238 tons in 2014 [2]. The decline in crop yields can be caused by erratic climates and pests and diseases. The type of virus that is reported to infect papaya plants is Papaya Ringspot Virus type P (PRSV-P). In the report of [3], papaya production can experience a yield reduction of up to 85-90% due to the attack of PRSV. PRSV is transmitted by aphids in a non-persistent manner [4], so as to allow rapid and widespread virus spread [5]; [6]. PRSV was first discovered in early 2012 in Aceh Besar District, Nanggroe Aceh Darussalam Province [7]. In 2013 by Medan Quarantine Class II Medan, Kualanamu has detected a PRSV in Namu Mbelin village, Namu Rambe sub-district, Deli Serdang Regency, [8]. In the Minister of Agriculture Regulation No. 31 / Permentan / KR.010 / 7/2018, PRSV is included as OPTK A2 Group I, whose existence is still limited in Indonesia so it needs to be watched out [9].

One of the sensitive, accurate and applicable virus detection methods is the DAS-ELISA (Double Antibody Sandwich-Enzyme-Linked Immunosorbent Assay) serology method [10]. The advantages of the DAS-ELISA method can detect viruses in very low concentrations (1-10ug / ml), need only a few
antibodies, testing can be done on rough sap plants, can be done in a mass, and can be done quantitative using Elisa Reader [11]. This study aims to detect PRSV in papaya plants in papaya plantations in North Sumatra.

2. Material and method

2.1. Survey
The survey was conducted in three districts, namely: (I) Deli Serdang Regency (0 - 300 masl), (II) Dairi Regency (301-500 masl), and (III) Pakpak Bharat District (501 - 1,000 masl). Of each district's chosen three locations.

2.2. Symptom observations
About 5 plant samples were taken randomly. Papaya plants that show symptoms of PRSV were placed in a paper bag and stored in a cooler to be taken to the laboratory.

2.3. PRSV-P detection with DAS-ELISA Method
The leaves, papaya skin, and seeds of each sample were crushed using General extract buffer (GEB) (1: 10 / wet weight/volume). Then Sap was put into an Effendorf and centrifuged at 5,000 rpm for 5 minutes. Sap is stored at 4ºC until it was used.

Micro plates are prepared with a map. The microplate well was filled with 100 ul PRSV antibodies each with a 1: 100 layer buffer, covered with aluminum foil, covered with wet paper, and stored in plastic. Micro plates were incubated at 4ºC for 24 hours. Next, empty the microplate then wash with PBST 2 times. Then dry the microplate by flipping and patting the surface covered by paper towel. Fill the microplate hole with sap sample of papaya plant that has been prepared as much as 100 µl per hole. Prepare positive control and negative control, the microplate is then stored in plastic that has been given a wet paper mat, then incubated at room temperature for 2 hours. The conjugate / ECI enzyme is prepared 10 minutes before the incubation time ends (the PRSV conjugate enzyme that has been dissolved by the buffer conjugate at a ratio of 1: 100).

Furthermore, the micro plates were washed 7 times with PBST. 100 ul of the conjugate enzyme was put into the plate hole. The dish was covered with wet tissue and incubated at room temperature for 2 hours. Then, the micro plates were washed 8 times with PBST. 100 ul PNP buffer (0.5 g PNP plus 5 ml PNP buffer) was added to the plate hole and incubated in a dark box at room temperature for 60 minutes.

Visual observations were made, the change in color of the liquid in the microplate hole to yellow showed a positive reaction. Quantitative analysis was performed with an ELISA Reader spectrophotometer with a wavelength of 405 nm. A positive result if the sample value is more than 2 times the average value of the negative control.

3. Results and discussion
Based on field observations, many papaya plants show symptoms of PRSV. The observations showed that the symptoms of PRSV in papaya plants varied. Diseased plants symptoms on the leaves were malformed, mosaic, mottling, the leaves become yellowed striped and there is bleaching of young leaves. In the fruit, there were concentric ring spots (ringspot), dark green spots on the fruit.

In papaya plants in Hamparan Perak Village Hamparan Perak District Deli Serdang Regency (Figure 1a), Saentis Village Percut Sei District Tuan Deli Serdang Regency (Figure 1b), Patumbak I Village Patumbak District Deli Serdang Regency (Figure 1c), Lau Njuhar Village Tanah Pinem District Dairi Regency (Figure 2a), Lau Baleng Village Lau Baleng District Dairi District (Figure 2b), Village: Sitinjo 1 Sitinjo District Dairi Regency (Figure 2c), Boang Manalu Village Salak District Pakpak Bharat Regency (Figure 3a), Siempat Rube Village Siempat Rube I District Pakpak Bharat Regency (Figure 3b), Kutadame Village Royal District Pakpak Bharat Regency (Figure 3c) were seen that the fruits were clearly marked with leaves showing striped symptoms, dark green mosaics such as blisters and malformations. On the stems and stems visible symptoms of streak and dark green lines.
### 3.1 Sympton in Deli Serdang Regency

**Figure 1a.** Hamparan Perak Village Hamparan Perak District Deli Serdang Regency  
a. Ring-shaped spot symptoms with dark green color,  
b. Symptoms of yellowish green mosaic and mosaics such as blisters on the leaves,  
c. Symptoms of steak and dark green lines on the stalk

**Figure 1b.** Saentis Village, Percut Sei District, Deli Serdang Regency  
a. Ring-shaped spot symptoms with dark green color,  
b. Symptoms of mottling and mosaic on the leaves,  
c. Symptoms of streaks and dark green lines on the stems and stems

**Figure 1c.** Patumbak I Village, Patumbak District, Deli Serdang Regency  
a. Ring-shaped spot symptoms are unclear and few,  
b. Symptoms of striped, dark green mosaics such as blisters and leaf formation,  
c. Symptoms of streaks and dark green lines on the stems and stems
3.2 Sympton in Dairi Regency

**Figure 2a.** Lau Njuhar Village, Tanah Pinem District, Dairi Regency
a. ring-shaped spot symptoms with a dark green color,
b. Symptoms of mottles and dark green mosaics such as blisters on leaves,
c. Symptoms of streaks and dark green lines on the stalk

**Figure 2b.** Lau Baleng Village, Lau Baleng District, Dairi Regency
a. Ring-shaped spot symptoms with dark green color,
b. Streaks and mozaic on the leaves, streaks and dark green lines on the stems

c. Symptoms of streaks and dark green lines on the stems and stems

**Figure 2c.** Village: Sitinjo 1 Sitinjo District Dairi Regency
a. Ring-shaped spot symptoms are clear and numerous,
b. Symptoms of striped, dark green mosaics such as blisters and leaf formation,
c. Symptoms of streaks and dark green lines on the stems and stems
3.3 Symptom in Pakpak Bharat Regency

Figure 3a. Boang Manalu Village, Salak District, Pakpak Bharat Regency
a. Ring-shaped spot symptoms with dark green color,
b. Symptoms of streaks and dark green lines on the stalk
c. Symptoms of mottles and dark green mosaics such as blisters on the leaves

Figure 3b. Siempat Rube Village Siempat Rube I District Pakpak Bharat Regency
a. Ring-shaped spot symptoms with dark green color,
b. Symptoms of streaks and dark green lines on the stems and stems,
c. Symptoms of mottling and mosaic on the leaves

Figure 3c. Kutadame Village, Royal District, Pakpak Bharat Regency
a. Ring-shaped spot symptoms are clear and numerous,
b. Symptoms of striped, dark green mosaics such as blisters and leaf formation,
c. Symptoms of streaks and dark green lines on the stems and stems

Variations in symptoms of plants infected with viruses are influenced by several factors, namely plant age, cultivars, plant genotypes, and plant growth phases. Other factors that influence symptoms of viral infection are environmental factors, including soil fertility and climate [12]. On papaya plants in Kutadame Village, Royal District, Pakpak Bharat Regency, there are small ring-shaped spots with a very large dark green color, on leaves, there are spots and mosaic symptoms and malformations and
malformations and on stems and stems. symptoms of streaks and dark green lines (Fig. 3c). The same symptoms on papaya plants infected with PRSV were also reported by [13] on leaves with mottled symptoms, [14] leaf abnormalities, [15] scratches and dark green symptoms on the stalks, on the fruit the ring-shaped spots appear in green dark.

The ELISA watershed method is widely used to detect various plant viruses because it is very sensitive [16]. The ELISA method is one of the most popular serology techniques because it is not only sensitive but also easy to do, accurate, simple, and does not require high costs. The results of the DAS-ELISA test were also measured quantitatively using an ELISA reader with a spectrophotometer with a wavelength of 405 nm (Table 1).

Table 1. DAS ELISA test on papaya plants

| No | Location | Leaves | Fruit Skin | Seeds |
|----|----------|--------|------------|-------|
|    |          | D1     | D2 | D3 | D4 | D5 | Kb1 | Kb2 | Kb3 | Kb4 | Kb5 | B1 | B2 | B3 | B4 | B5 |
| .1a | Hamparan Perak Village, Hamparan Perak District, Deli Serdang Regency | + | + | + | + | + | + | + | + | - | - | - | - | - | - | - |
| .1b | Saentis Village, Perceut Sei District, Deli Serdang Regency | + | + | + | + | + | + | + | + | - | - | - | - | - | - | - |
| .1c | Patumbak I Village, Patumbak District, Deli Serdang Regency | + | + | + | + | + | + | + | + | - | - | - | - | - | - | - |
| .2a | Lau Njuhar Village, Tanah Pinem District, Dairi Regency | + | + | + | + | + | + | + | + | - | - | - | - | - | - | - |
| .2b | Lau Baleng Village, Lau Baleng District, Dairi Regency | + | + | + | + | + | + | + | + | - | - | - | - | - | - | - |
| .2c | Village: Sitinjo 1 Sitinjo District Dairi Regency | + | + | + | + | + | + | + | + | - | - | - | - | - | - | - |
| .3a | Boang Manalu Village, Salak District, Pakpak Bharat Regency | + | + | + | + | + | + | + | + | - | - | - | - | - | - | - |
| .3b | Siempat Rube Village, Siempat Rube I District Pakpak Bharat Regency | + | + | + | + | + | + | + | + | - | - | - | - | - | - | - |
| .3c | Kutadame Village, Royal District, Pakpak Bharat Regency | + | + | + | + | + | + | + | + | - | - | - | - | - | - | - |

Note: D = Papaya leaves; Kb = Papaya fruit skin; B = Papaya fruit seeds

A total of 135 samples (leaves, papaya rind, and seeds) were obtained from 9 papaya plantations (Table 1). Based on the DAS ELISA test, papaya leaf samples and papaya rind at all study sites showed positive results against the PRSV-P antibody, this indicates that they were infected with PRSV-P. According to [17], virulence is one of the factors that influence the development of pathogens and symptoms, isolates with high virulence can cause very severe symptoms in infected plants, whereas isolates with low virulence often do not cause symptoms or only cause mild symptoms.

Seed samples at all study sites showed negative results on PRSV. In the Philippines showed that only 2 of 1335 seeds (0.15%) of Cavite papaya that growth from PRSV-infected seeds showed symptoms such as PRSV. In other studies, there were no viruses transmitted by seeds detected in papaya [18]. From this study, it was confirmed that PRSV was not carried by seed. The virus will easily spread because these fleas can fly and move around easily. In addition, his lightweight body will easily follow the direction of the wind. Therefore, it is not surprising that the power of attack and the spread of lice is very fast. If one plant has been attacked by a virus, its transmission to other plants will not require a long time, even though the distance between plants is quite far.
4. Conclusion
Papaya Ringspot Virus - P has been detected in Hamparan Perak Village, Pond Village, Percut, Patumbak Duap Village, Lau Njuhar Village, Lau Balang Village, Village: Sitinjo 1, Boang Manalu, Siempat Rube, Kutadame Village. Serology detection using the DAS-ELISA method showed that papaya leaf and papaya bind were positively infected with the PRSV, but on papaya seeds at all study sites was negative.

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